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A New System of Anatomy

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This book has been specially written as a practical guide to the courses of regional anatomy that lead to the second M.B. examination. It gives a comprehensive picture of the anatomy of the entire body based on an extensive series of new dissections specially made for the purpose.

The book has been written with the following considerations in mind:

1. The increasing volume of new knowledge makes it inevitable that some of the older parts of the curriculum must be shortened if the student is to have time for the many new subjects now included in the curriculum.
2. The average student needs to know only the essentials of anatomy but he should know them really well.
3. Topographical anatomy can only be learnt properly in the dissecting room and a book on practical anatomy should describe only what can be demonstrated there.
4. A practical book should be based on an extensive series of dissections made especially for the purpose and it should give instructions on dissection and directions on what to observe in sufficient detail to enable any student of average intelligence to complete his dissection without seeking help from the teaching staff.
5. Detail which has no scientific or educational value should be eliminated and time should not be wasted by students trying to reveal features that can only be demonstrated adequately in special preparations.
6. The primary purpose of a medical student's work in the dissecting room is to learn enough about the structure of the body to enable him to understand the practical applications that follow in his clinical course. It is not the function of a pre-clinical department to teach the applied anatomy which is better left to those whose concern it is.
7. A practical book should only include as much osteology as a student needs to understand how the soft parts of the body relate to its bony structure.

The illustrations, which number 540, are an outstanding feature, nearly all of them being photographs of actual dissections made especially for this book. They show all the structures described in the text with extreme clarity, and as they will be seen by the student in his own dissection. Great care has been taken not to overcrowd them with leader-lines and at times, to avoid this, the same illustration is repeated with different leader-lines attached. They have been carefully placed so that they are adjacent to the descriptive text and it is seldom necessary to turn a page to see the relevant illustration.

The nomenclature used is an English equivalent of the Paris *Nomina Anatomica* as agreed at the Sixth International Congress of Anatomists held in Paris in 1955, and revised at the Seventh Congress in New York in 1960. Where the English equivalent differs markedly from a literal translation of the official Latin term the Latin has been added in brackets in the text. In addition the Index provides a full glossary, the Latin P.N.A. terms being placed in a separate column adjacent to their English equivalents.

Guidance on the technique of dissection and on terminology is given in the Introduction and, for convenience, a brief statement on the layout of the lymphatic system has been included as an appendix.

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GALACTOSAEMIA

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1 Clinical manifestations
2 Morbid anatomy
3 Pathogenesis and biochemical lesion
4 Genetics
References

The realization that galactosaemia is not a clinical rarity—at least 50 cases have been diagnosed in the United Kingdom during the past few years—and the success in elucidating the underlying biochemical disorder, as well as the advances made in establishing the mode of inheritance, have earned it a special place among inborn errors of metabolism. Galactosaemia also illustrates very strikingly the interplay of inherited, endogenous factors and environment, since effective dietary control can assure physical health and normal mental development to those affected (Holzel, Komrower & Schwarz, 1957; Donnell, Bergren & Cleland, 1960).

1. Clinical Manifestations

The clinical manifestations of galactosaemia vary considerably in severity. In its severe form the disease becomes evident towards the end of the first week of life, a few days after milk feeding has been started. Listlessness, refusal to feed, and vomiting, are soon followed by loss of weight, enlargement of liver, jaundice and diarrhoea. The urine shows galactosuria, proteinuria and an abnormal aminoaciduria. Cataracts appear. A state of severe malnutrition ensues, with signs of gross liver damage, haemorrhages due to prothrombin deficiency, ascites and generalized oedema. Intercurrent infection or liver failure finally leads to death.

There are, however, a number of recorded cases of patients who have survived without treatment and in whom the disease took its natural course for a number of years before the diagnosis was established (Bray, Isaac & Watkins, 1952; Holzel, 1959). Cirrhosis of the liver, cataracts and mental retardation are common sequelae, but survival with normal intelligence was recorded in two brothers aged 8 years and 14 years respectively (Durand & Semach, 1955), and in one patient aged 65 years (Hugh-Jones, Newcomb & Hsia, 1960).

Apart from the suggestive clinical features, the diagnosis rests largely on laboratory evidence. Whilst the child is on a milk diet there is a high level of blood galactose and simultaneously a marked lowering of blood glucose (Mason & Turner, 1935; Bruck & Rapoport, 1945). Galactose in the urine is identified by chemical and chromatographic methods. The aminoaciduria (Bickel & Hickmans, 1952; Holzel, Komrower & Wilson, 1952) is generally encountered only in the graver forms of the disorder.[1] There is a predominance of the neutral aliphatic acids. Komrower (1953) and Hsia, Hsia, Green, Kay & Gellis (1954) found normal α-amino nitrogen levels in the serum, and Cusworth, Dent & Flynn (1955) were able to show that the aminoaciduria was due to a disturbance of tubular reabsorption. Hyperchloraemic acidosis as an expression of renal tubular dysfunction has also been noticed (Berger, 1955; Komrower, Schwarz, Holzel & Golberg, 1956). Complete removal of galactose from the diet leads to the disappearance of the aminoaciduria within five to seven days; its persistence is an indication of incomplete withdrawal of galactose from the diet (Holzel *et al.* 1957). The galactose tolerance test is a somewhat dangerous diagnostic tool, particularly in the seriously ill patient, because of the severe hypoglycaemia it may induce. Ultimately the diagnosis rests on the demonstration of accumulated galactose 1-phosphate in the red cells (Schwarz, Holzel & Komrower, 1958) and the deficiency of galactose 1-phosphate uridyltransferase (Kalckar, Anderson & Isselbacher, 1956). The last two tests can be used to establish the diagnosis at birth.

[1] See also Woolf, p. 224 of this number of the Bulletin.—Ed.

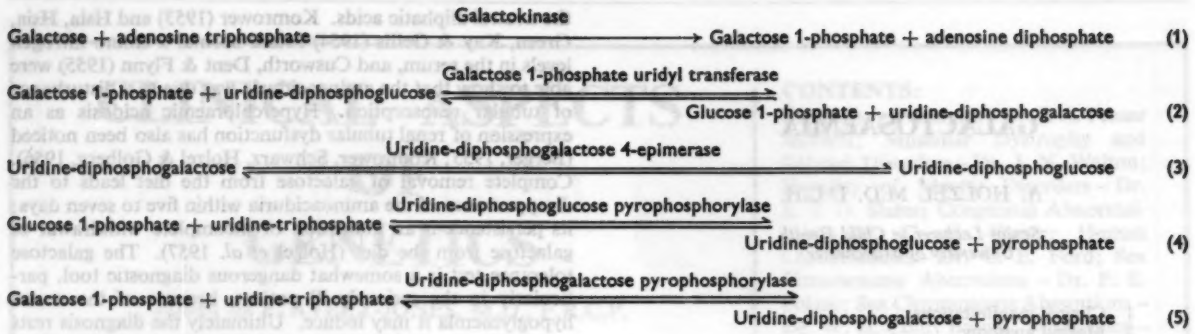
2. Morbid Anatomy

The liver, kidney, lens and brain are the organs most likely to suffer but, apart from cataracts, the anatomical changes are almost exclusively limited to the liver. Townsend, Mason & Strong (1951) pointed to the similarity with those found in Laennec's cirrhosis. There is distorting of the lobular structure, fibrosis, bile-duct proliferation and a pseudo-acinar, alveolar pattern which has been regarded as characteristic. At an early stage these changes are reversible.

3. Pathogenesis and Biochemical Lesion

In 1935 Mason & Turner advanced the hypothesis of relative glucose starvation as a cause of the clinical manifestations in galactosaemia. Bruck & Rapoport (1945) postulated a direct toxic effect of galactose, whilst Craig & Maddock (1953), on the basis of experimental studies, attributed it to protein deficiency. In 1955 Schwarz, Golberg, Komrower & Holzel showed that the erythrocytes of galactosaemic children accumulated galactose 1-phosphate when their diet contained milk, or when such cells were exposed to galactose in vitro. Concomitant with the accumulation of galactose 1-phosphate, there was also a partial inhibition of oxygen uptake and a reduction in ester phosphate in the galactosaemic cells (Schwarz, Golberg, Komrower & Holzel, 1956).

Galactose 1-phosphate was also found in the liver, kidney, brain, tongue, adrenals and heart of two infants who died shortly after the diagnosis of galactosaemia had been made (Schwarz, 1960). It was discovered, too, in the capsules of cataractous lenses from rats fed on a high galactose diet (Schwarz & Golberg, 1955). The discovery of galactose 1-phosphate in the galactosaemic cells, and later on in the tissues, indicated the block in the metabolic pathway of galactose. Kalckar *et al.* (1956) confirmed these findings and were further able to show that galactosaemia was due to a congenital defect in a nucleotide transferase, namely galactose 1-phosphate uridyl transferase, whilst other enzymes necessary for galactose metabolism were present in normal quantities. Leloir (1951) and Kalckar, Braganca & Munch-Petersen (1953) contributed a great deal to the clarification of the enzymic reactions involved in the conversion of galactose into glucose. Five steps have been recognized in this process.


Galactokinase
Galactose + adenosine triphosphate → Galactose 1-phosphate + adenosine diphosphate (1)
Galactose 1-phosphate uridyl transferase
Galactose 1-phosphate + uridine-diphosphoglucose ⇌ Glucose 1-phosphate + uridine-diphosphogalactose (2)
Uridine-diphosphogalactose 4-epimerase
Uridine-diphosphogalactose ⇌ Uridine-diphosphoglucose (3)
Uridine-diphosphoglucose pyrophosphorylase
Glucose 1-phosphate + uridine-triphosphate ⇌ Uridine-diphosphoglucose + pyrophosphate (4)
Uridine-diphosphogalactose pyrophosphorylase
Galactose 1-phosphate + uridine-triphosphate ⇌ Uridine-diphosphogalactose + pyrophosphate (5)

In the first reaction galactose is phosphorylated by the enzyme galactokinase to galactose 1-phosphate with the aid of adenosine triphosphate. This process is not reversible. In the second stage galactose 1-phosphate is normally converted to glucose 1-phosphate, involving the nucleotide uridine-diphosphoglucose (UDPG) as a specific co-factor, and uridine-diphosphogalactose (UDPGal) is formed, which supplies the galactose essential for galactolipids, mucopolysaccharides and other galactose-containing compounds. This reaction is catalysed by the enzyme galactose 1-phosphate uridyl transferase, and is reversible. However, in its absence the resulting metabolic block leads to the accumulation of galactose 1-phosphate and interferes with the utilization of exogenous galactose. In step three UDPGal is directly converted into UDPG. The enzyme involved is UDPGal 4-epimerase, and diphosphopyridine nucleotide is necessary as a co-factor. This, too, is a reversible reaction, and UDPGal can therefore be formed from UDPG, which in turn can be synthesized from glucose 1-phosphate in stage four. These reactions offer an explanation why the body can satisfy its needs for galactose even on a galactose-free diet. Isselbacher's discovery (1958) of a uridine-diphosphogalactose pyrophosphorylase in mammalian brain and liver presents the possibility of an alternative pathway by which galactose 1-phosphate can be metabolized further, as set out in step five. The activity of this enzyme is very weak in foetal and neonatal tissue but tends to increase with age, although it never exceeds more than approximately one-sixth of galactose 1-phosphate uridyl transferase action. Activation of the UDPGal pyrophosphorylase may be one reason why certain disease processes in galactosaemia seem to come to a spontaneous arrest. It has already been stated that the intracellular accumulation of galactose 1-phosphate leads to an appreciable reduction of the respiratory rate of erythrocytes, and it is a justifiable assumption that other tissue cells are similarly affected. Maximal galactose phosphate concentrations in red cells in untreated patients ranged from 300 to 400 mg./ml. (Schwarz, 1960), and such levels were reached within a relatively short time. A minimal toxic level has not yet been ascertained. In a number of studies recently published it was demonstrated that galactose 1-phosphate can inhibit such enzymes as phosphoglucomutase, glucose 6-phosphate phosphatase and dehydrogenase (Ginsburg & Neufeld, 1957; Sidbury, 1957; Lerman, 1960) and in this way can derange normal metabolism. This effect is not limited to human cells, since Kurahashi & Wahba (1958) found that accumulation of galactose 1-phosphate in a mutant of *Escherichia coli* lacking in uridyl transferase led to a prolonged lag phase before the onset of growth when the culture was incubated with galactose.

As far as bacteria are concerned, Kurahashi (1957) and Morse, Lederberg & Lederberg (1956) have shown that four different mutants of *E. coli* K-12 lacking in uridyl transferase had their defect in different genes located in close proximity to each other. It is possible that this may also apply to man, but it would be impossible to detect such close linkage except in bacteria.

4. Genetics

The relatively high incidence of galactosaemia in siblings, and in the offspring of consanguineous marriages, as well as the more or less equal occurrence of the condition in both sexes and its absence in the parents, suggests that it might be transmitted by a single autosomal recessive gene. Manifest galactosaemia in more than one member of a sibship was reported by Göppert (1917), Bell, Blair, Lindsay & Watson (1950), Donnell & Lann (1951), Gorter (1951), Townsend *et al.* (1951), Brodie (1952), LoPresti, Itani & Rice (1952), Cox & Pugh (1954), Hudson, Ireland, Ockenden & White-Jones (1954), Durand & Semach (1955), Holzel & Komrower (1955) and Bain, Bowden, Chute, Jackson, Sass-Kortsak & Walker (1957). Consanguinity in the parents was noted by Lodding (1954), Flury & Berger (1955) and Holzel & Komrower (1955). Hugh-Jones *et al.* (1960), in an analysis of the data published in the literature on galactosaemia, found in the total series, where the sex had been recorded, that there were 66 males to 40 females. According to his calculations, there was no statistical evidence that the difference in sex incidence was of significance as compared with the sex ratio in a large published series of normal children from Birmingham (Crosse, 1949).

As no studies on the genetics of galactosaemia had previously been undertaken, Holzel & Komrower (1955) used the galactose tolerance test to detect the heterozygous carriers. They investigated 20 members of five families in which cases of galactosaemia had occurred. In only one family was reduced tolerance for galactose obvious in both parents, whilst in the remaining four families only one parent showed an abnormal response. However, abnormal test results were also found in some of the siblings of the patients and other relatives. But the difficulties inherent in such a relatively crude laboratory method, particularly variation in the rate of intestinal absorption, have to be borne in mind. More recently, Donnell, Bergren & Roldan (1959) attempted to re-evaluate

the oral galactose tolerance test as a means of identifying the heterozygous state in 39 members of 13 families with 21 galactosaemic children. They used 35 g./m.² of body-surface and modified the galactose index[1] by including the three-hour values. Although on a statistical basis the group of fathers, mothers and siblings of affected children could be clearly separated from normal persons, the overlap of individual values was so considerable that the technique has to be regarded as unsuitable for the definition of the heterozygous carrier. Hsia, Huang & Driscoll (1958), employing the method developed by Kalckar and his associates for the determination of galactose 1-phosphate uridyl transferase in five families, found a definite difference between the mean values of heterozygotes and normal controls, but again the overlap in the individual cases was too great to allow precise interpretation. Kirkman & Bynum (1959) were able to achieve a greater degree of accuracy by a different technique. This involved measuring, by the Warburg manometric technique, the oxygen consumption of haemolysates (to which various co-factors had been added) on galactose 1-phosphate substrate. Galactose 1-phosphate uridyl transferase activity was expressed as microlitres of oxygen uptake per hour for 0.3 ml. packed-cell haemolysate. The blood of 18 parents of galactosaemic children was compared with blood samples of 18 normal controls. The parents came from ten independent families. The following results were obtained:

arithmetic mean for parents: 20.2 ± 1.2 (± S.E.);
arithmetic mean for patients: 5.09 ± 0.85 (± S.E.);
arithmetic mean for normal subjects: 31.8 ± 1.0 (± S.E.).

There was practically no activity in the blood of the galactosaemic children tested, whilst the parents, although apparently healthy, showed approximately 64% of the activity of the normal subjects.

Donnell, Bergren, Bretthauer & Hansen (1960) employed a modification (see Bretthauer, Hansen, Donnell & Bergren, 1959) of Kalckar's original method of enzyme assay. By using different concentrations of substrate, and altering the ratio of substrate to enzyme, they achieved a more sensitive quantitative technique. Basically it still depends on the principle underlying the method of Kalckar *et al.* (1956); i.e., the estimation of UDPG utilized, and the amount of UDPGal formed on incubation of a red-cell haemolysate with appropriate amounts of substrate.

Galactose 1-phosphate uridyl transferase activity in the erythrocytes of 106 normal controls (67 adults and 39 children) ranged from 3.8 to 8.3 μmole UDPG/ml. erythrocytes /hour, with a mean of 5.9 and S.D. of ±1.01. The mean value of enzyme activity in 13 galactosaemic children was 0.1; in their parents, 2.9 ± 0.83; in the 11 siblings assumed to be heterozygous the mean was 3.0 ± 0.87; and in the six siblings regarded as healthy it was 6.0 ± 0.78. Extending the enzyme assay to 117 relatives of the affected children, it was found that they fell into two groups, if the value of 4 μmole UDPG/ml. erythrocytes/hour was taken as an arbitrary dividing line. This value was chosen because enzyme activity ascertained in the parents was below that level. The mean value in one group consisting of 39 members was 2.8 μmole UDPG/ml. erythrocytes/hour, with S.D. ±0.70; these persons were classed as heterozygotes and in the second group of 78 relatives the mean was 5.7 ± 0.99; these were regarded as normal.

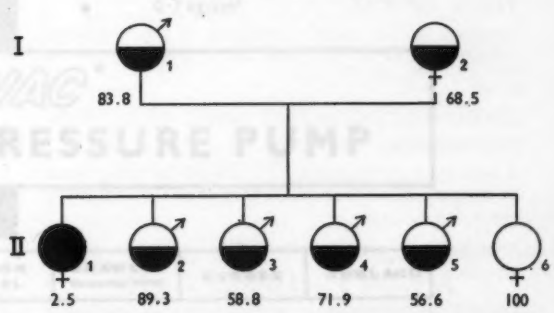
Hsia, Tannenbaum, Schneider, Huang & Simpson (1960) measured uridyl transferase activity in the red cells of the members of 14 galactosaemic families, using four different methods, two based on UDPG consumption and two upon manometric estimation of oxygen uptake. Hsia *et al.* (1960) claim that small amounts of uridyl transferase activity were observed with all four methods in a number of the galactosaemic homozygotes. The data gained with this battery of techniques confirm that the mean values for the heterozygotes were significantly lower than for a group of normal subjects serving as controls. In the hands of these workers the manometric methods measuring oxygen uptake reduce the overlap between normal subjects and heterozygotes to a minimum, irrespective of supplementation with phosphoglucomutase and glucose 1 : 6-diphosphate.

In an investigation of 18 galactosaemic families, Schwarz, Wells, Holzel and Komrower (unpublished), with a modification of manometric method (see Kirkman & Bynum, 1959), expressed the oxygen uptake on galactose 1-phosphate substrate as a percentage of the oxygen uptake on glucose 1-phosphate. The arithmetic mean value for galactosaemic patients was 5% and that for the parents 68% of that in unrelated normal controls.

Values of uridyl transferase activity in family R (fig. 1) may serve as an example. Only minimal activity was found in II-1, the patient with galactosaemia. Three of the siblings (II-3, II-4, and II-5) are clearly separable as presumptive heterozygotes. A fourth, II-6, is almost certainly homozygous for the normal gene. The fifth sibling (II-2) and the father (I-1) are in the range where it is not possible to be sure that they are heterozygotes. The mother (I-2) is probably heterozygous.

It is not known if the lack of activity of galactose 1-phosphate uridyl transferase in patients suffering from galactosaemia is due to the absence of the enzyme or, as is more probable, to the production of a structurally different substance which is inactive. Although the enzyme deficiency has been detected in every case of clinical galactosaemia examined, this does not necessarily prove that we are dealing with an identical biochemical lesion in all such cases.

FIG. 1. Inheritance of Uridyl Transferase Activity in Family R



Variations are expressed in percentages of "normal", i.e., the mean value in control subjects not related to galactosaemic subjects.

[1] The galactose index is usually defined as the sum of half-hourly blood galactose values up to two hours, expressed in mg./100 ml.

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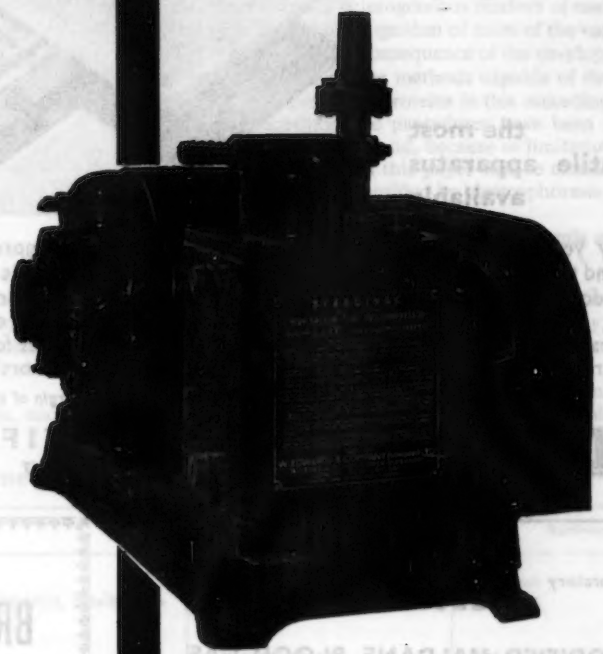

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INHERITED VARIATIONS OF HUMAN PLASMA PROTEINS

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1 Analbuminaemia, afibrinogenaemia and agammaglobulinaemia
2 The haptoglobins
3 The transferrins
4 Double albumin
5 A new "albumin-like" protein
6 Discussion
References

One of the most interesting developments in human genetics in recent years has been the discovery of a whole series of genetically determined variations in the formation of different plasma proteins. A list of some of these is given in Table I. It illustrates something of the diversity of phenomena which these variations represent, and also the variety of techniques which have been used in their detection.

Some of these peculiarities in protein synthesis, such as afibrinogenaemia, agammaglobulinaemia, caeruloplasmin deficiency and hypophosphatasia, give rise to, or are associated with, characteristic clinical syndromes. They occur only rarely, and one has little hesitation in regarding the affected individuals as "abnormal". Other types of variation, however, result in no obvious differences in health or fitness. Each of the haptoglobin phenotypes, for example, may be found quite commonly among apparently normal and healthy individuals. The differences in protein synthesis, which these various phenotypes reflect, can therefore only be regarded as different versions of "normality".

Plasma contains a heterogeneous mixture of many different protein species. The recognition of most of the variants listed in Table I has been the consequence of the development in the last few years of sensitive methods capable of detecting and characterizing individual proteins in this exceedingly complex mixture. Electrophoretic procedures have been particularly important in this respect and, because of limitations of space, the variants discussed in this paper will be confined to those which can be readily identified by electrophoresis.

1. Analbuminaemia, Afibrinogenaemia and Agammaglobulinaemia

In certain instances, individuals with a particular genetic constitution are completely, or almost completely, deficient in one of the major fractions observed in the electrophoresis of human plasma by classical methods.

One such peculiarity is analbuminaemia (Bennhold, Peters & Roth, 1954; Bennhold, 1956). This was first discovered in

TABLE I. Some Plasma Protein Variants in Man

Protein	Variants	Method of detection	References
Albumin	Analbuminaemia	Electrophoresis	See text
	"Double albumin" (paralbuminaemia, bisalbuminaemia)	Electrophoresis	See text
Fibrinogen	Afibrinogenaemia	Electrophoresis	See text
Haptoglobin	Various phenotypes: 1-1, 2-1, 2-2, 2-1 modified, 0, etc.	Starch-gel electrophoresis	See text
Transferrin	C, B_0, B_1, B_2, D_0, D_1, D_2, D_3, D_4	Starch-gel electrophoresis	See text
γ-Globulin	Agammaglobulinaemia	Electrophoresis	See text
	Gm types: Gm (a+), Gm (a−), Gm (b+), etc.	Serological	Grubb (1959)
Caeruloplasmin	Deficiency in Wilson's disease	Immunochemical and spectrophotometric	Scheinberg & Gitlin (1952)
Alkaline phosphatase	Deficiency in hypophosphatasia	Enzymic	Rathbun (1948); Fraser (1957)
Serum cholinesterase	Atypical form of enzyme	Enzymic	Kalow (1959); Lehmann, Silk & Liddell (1961)
? Albumin	New "albumin-like" component present in sera of several related individuals	Two-dimensional paper and starch-gel electrophoresis	See text
β-Lipoprotein	Absence or partial deficiency	Electrophoresis and ultracentrifuge	Salt, Wolff, Lloyd, Fosbrooke, Cameron & Hubble (1960)
α_2- Component	Three phenotypes: 1, 2, 3	Immunoelectrophoresis	Hirschfeld (1959); Hirschfeld, Jonsson & Rasmuson (1960)
Coagulation factors	Deficiencies of antihaemophilic globulin, Christmas factor, Factor V, etc.	Coagulation tests	Biggs & Macfarlane (1957)

a 31-year-old female farm worker who complained of tiring easily, and who had very slight oedema. Family studies led to the discovery that her brother had precisely the same plasma protein abnormality, although he felt quite fit and was able to do heavy work on the land. In neither of these individuals could serum albumin be detected electrophoretically. Immunochemical studies subsequently indicated that a trace of serum albumin was probably present, though this could amount to no more than about $\frac{1}{2,000}$ of that normally found. A large number of other relatives were examined, but no abnormality was detected. The parents of the two affected individuals were second cousins. It is likely that the abnormality is determined by a rare mutant gene for which the affected individuals are homozygous. The level of serum albumin appeared normal in the presumed heterozygotes.

Afibrinogenaemia illustrates an analogous type of defect. Here, no fibrinogen can be detected electrophoretically although, again, immunochemical measurements show that very small amounts (about 1 mg./100 ml.) may actually be present (Gitlin & Borges, 1953). The affected subjects suffer from severe haemorrhages, and usually die in early life. They appear to be homozygous for a rare autosomal gene. There is some evidence that the heterozygotes may have a slightly lower concentration of plasma fibrinogen than normal people (Frick & McQuarrie, 1954), but this does not lead to any ill effects.

Agammaglobulinaemia represents another, though probably somewhat more complex, example of the same kind of phenomenon. In the congenital type of this condition, little or no γ-globulin is found electrophoretically. Immunochemical methods indicate that in most cases less than about 25 mg./100 ml. are present (Gitlin, 1955). Besides this, studies by immunoelectrophoresis have shown that at least two proteins that normally form part of the β-globulin fraction, and seem to be immunochemically distinct from γ-globulin, are also deficient (Gitlin, Hitzig & Janeway, 1956). Persons so affected exhibit a gross defect in the ability to elaborate antibodies. The genetic evidence suggests that, at least in some cases, the abnormality is determined by a rare sex-linked and apparently "recessive" gene (Gitlin & Janeway, 1960).

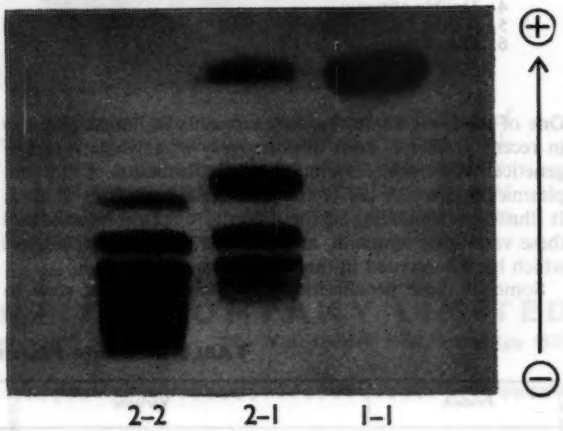
In each of these three conditions the deficiency of a particular protein has been shown by infusion studies to be due to a failure in synthesis, and not to an excessive rate of destruction. Another important point is that in none of these cases is there any indication that an unusual kind of protein is formed in place of the deficient one. Finally, it should be noted that in each condition the deficiency in protein synthesis is a highly specific one.

These examples illustrate how certain genes may result in the failure to synthesize particular proteins at the normal rate. It is of interest to contrast this with the situation exemplified by the haptoglobin and transferrin variants where allelic genes lead to the synthesis of qualitatively different forms of the protein, at roughly similar rates.

2. The Haptoglobins

When plasmas or sera from normal individuals of European stock are subjected to starch-gel electrophoresis, three distinct types of protein pattern can be readily recognized (Smithies, 1955). The differences are due to variation in a group of proteins known as the haptoglobins, which in conventional electrophoresis form part of the α_2-globulin fraction. They possess the distinctive property of complexing with haemoglobin; and this is very useful in identifying the types because, if some haemoglobin is added to the plasma before electrophoresis, the haptoglobin–haemoglobin complexes that are formed migrate in a characteristic manner and can be detected specifically by reagents such as benzidine, which do not react with other plasma proteins present (fig. 1). Only a small amount of haemoglobin (about 100–150 mg./100 ml.) is usually required to saturate the binding capacity of the haptoglobins in normal plasma, and any excess haptoglobin migrates independently in the free state.

FIG. 1. Starch-Gel Electrophoresis, Showing Haptoglobin-Haemoglobin Complexes of the Three Phenotypes, 2–2, 2–1 and 1–1, Stained with a Benzidine Reagent (pH 8.6)



The three haptoglobin phenotypes are now referred to as 1-1, 2-1, 2-2, and in European populations occur with a frequency of about 0.16, 0.48 and 0.36 respectively. Only a single haptoglobin component can be detected in the 1-1 phenotype. A much smaller amount of a component with the same mobility can be detected in the 2-1 phenotype, but none in the 2-2 phenotype. However, both the 2-1 and the 2-2 phenotypes show a series of components moving more slowly than the 1-1 component, and it is of some significance that the mobility of each of the components in the 2-1 phenotype is different from that of any in the 2-2 phenotype. Thus the haptoglobin pattern is qualitatively distinct in the three types of individuals, and simple mixing of the 1-1 and 2-2 plasmas will not produce a pattern corresponding to the 2-1 phenotype.

The multiple protein zones seen in starch-gel electrophoresis of the 2-1 and 2-2 haptoglobin phenotypes probably each represent a series of polymers of increasing molecular weight. The pattern can be considerably simplified after treatment of the proteins with reagents which cause a reductive cleavage of disulphide bonds (Smithies & Connell, 1959; Smithies, 1960). After such treatment the protein sub-units derived from the 2-1 phenotype appear to represent a simple mixture of those obtained from the 2-2 and 1-1 phenotypes.

The haptoglobin types are characteristic of the individuals, and are inherited. Smithies & Walker (1955) suggested that they are determined by a pair of allelic genes. These are termed Hp^1 and Hp^2, 1-1 individuals being homozygous for

Hp^1, 2-2 individuals being homozygous for Hp^2, and 2-1 individuals being heterozygous for Hp^1 and Hp^2. The hypothesis requires that no 2-2 individuals should occur among the offspring of a 1-1 parent, no 1-1 individuals should occur among the offspring of a 2-2 parent, and that matings 1-1 × 2-2 should result in only 2-1 offspring. Extensive family studies (Smithies & Walker, 1955; Galatius-Jensen, 1958a; Harris, Robson & Siniscalco, 1959) are in substantial agreement with these expectations, although some rare exceptions that will be referred to later have been noted.

The genetic hypothesis may also be tested by comparing the observed frequencies of the three phenotypes in different populations with the expected frequency, assuming a Hardy-Weinberg equilibrium. This can be done without making further assumptions only in those populations where virtually all individuals may be classified into one or other of the three phenotypes. This calculation is shown in Table II for various population samples, and it will be seen that there is good agreement between the observed and the expected figures.

In European populations occasional individuals are found (less than 1% in most random surveys) in whom no haptoglobins can be detected in the plasma. A much higher incidence is often found in African populations (Allison, Blumberg & ap Rees, 1958). Whether or not haptoglobins are detected when they are present in very small amounts will, of course, depend on the technical procedures used. However, in most cases which have been described as ahaptoglobinaemia or the O phenotype, any haptoglobin present was probably less than 5% of the quantity usually found. Such a situation could arise because of an excessively rapid rate of removal of haptoglobin from the circulation, or because of defective synthesis. Both these causes probably operate in different cases.

One way in which plasma haptoglobin can be depleted is by haemolysis. Laurell & Nyman (1957) have shown that, when haemoglobin is infused into an individual, the haptoglobin–haemoglobin complexes formed are rapidly removed from the circulation and the plasma may be completely depleted of haptoglobin within a few hours. When the haemoglobin infusion has ceased, fresh haptoglobin begins to appear in the circulation and eventually reaches its original level. A similar phenomenon occurs in haemolytic disease (Nosslin & Nyman, 1958). For example, patients with untreated pernicious anaemia show no haptoglobins, but these appear within a few days of treatment with vitamin B_{12} and reach a normal concentration within 2–3 weeks (Nyman, 1957).

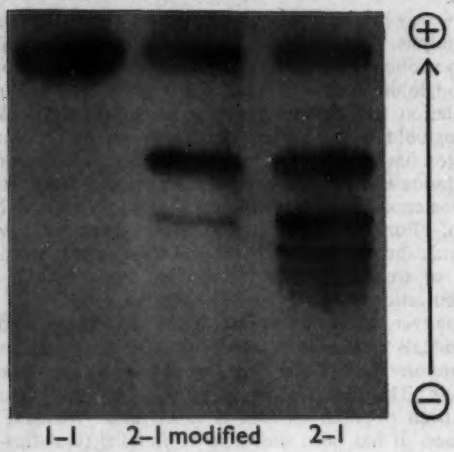
However, there seems no doubt that there also occur individuals with no detectable haptoglobins or with only very minute amounts of them, in the absence of any haemolytic process. This phenomenon appears to be very uncommon in European populations, but is much more frequent among negroes. It has been shown to be familial (Galatius-Jensen, 1958b; Harris *et al.* 1959; Sutton, Neel, Livingstone, Binson, Kunstadter & Trombley, 1959; Giblett & Steinberg, 1960), but its precise genetic basis remains uncertain. The defect can perhaps be regarded as analogous to those defects occurring

TABLE II. Incidence of Haptoglobin Phenotypes in Different Populations, and the Expected Incidence, Assuming a Hardy-Weinberg Equilibrium

Population	Observed (O) / Expected (E)	Total	Haptoglobin types			Estimated gene frequencies	
			1-1	2-1	2-2	Hp^1	Hp^2
England	O	179	33	88	58	0.43	0.57
	E		33.1	87.7	58.2		
Northern Italy (Berra)	O	119	20	57	42	0.41	0.59
	E		20.0	57.6	41.4		
(Cologna)	O	208	23	99	86	0.35	0.65
	E		25.5	94.6	87.9		
Southern Italy (Naples)	O	93	10	44	39	0.34	0.66
	E		10.8	41.7	40.5		
Sardinia (Illoral)	O	147	18	74	55	0.37	0.63
	E		20.1	68.5	58.4		
Sicily (Catania)	O	107	16	53	38	0.40	0.60
	E		17.1	51.4	38.5		
Borneo	O	22	6	10	6	0.50	0.50
	E		5.5	11.0	5.5		
Persia	O	34	2	13	19	0.25	0.75
	E		2.1	12.8	19.1		
Japanese (in USA)	O	23	2	10	11	0.30	0.70
	E		2.0	9.7	11.3		
Totals	O	932	130	448	354		
	E		136.2	435.0	360.8		

From Harris, Robson & Siniscalco (1959) by permission of the Ciba Foundation

FIG. 2. Starch-Gel Electrophoresis, Showing Haptoglobin-Haemoglobin Complexes of the Phenotypes 1–1, 2–1 Modified, and 2–1, Stained with a Benzidine Reagent (pH 8.6)



in the conditions, afibrinogenaemia and analbuminaemia, discussed earlier.

In negro populations, yet another distinctive haptoglobin phenotype is not uncommonly found. This has been called the 2-1 modified phenotype (Connell & Smithies, 1959). It is extremely rare in people of European stock, but has been observed. The 2-1 modified phenotype resembles the ordinary 2-1 phenotype in that the mobilities of the haptoglobin components observed in starch-gel electrophoresis are apparently the same. It differs, however, in the relative concentrations of these components (fig. 2). There is relatively much more of the fast-moving component characteristic of the 1-1 phenotype, and relatively much less of the more slowly moving components typical of the 2-1 phenotype. It has been found with an incidence of about 10% in several negro populations, and has generally been about one-half to one-third as frequent as the ordinary 2-1 phenotype in these populations. The 2-1 modified phenotype is certainly genetically determined. The family data collected so far (Giblett & Steinberg, 1960; Harris, Lawler, Robson & Smithies, 1960) are consistent with the hypothesis that individuals of this phenotype are heterozygous for the Hp^1 gene, and for a further allele designated Hp^{1M}. However, the possibility that the modification in phenotype may be induced by a gene at another locus has not been completely excluded.

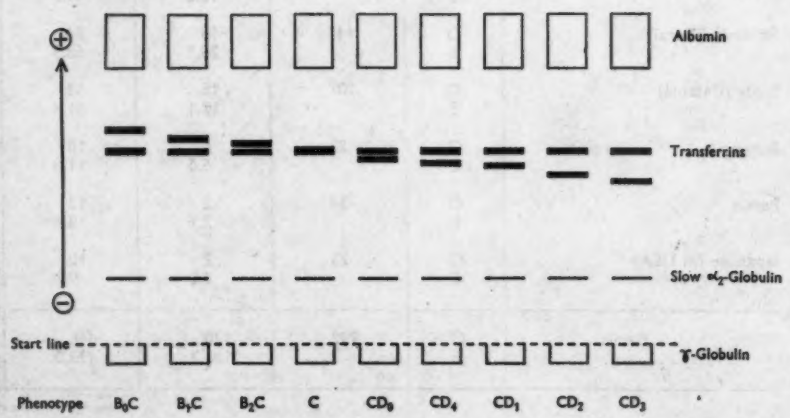
There are probably a number of other rare genes which can also influence, in various ways, the formation of haptoglobins. Giblett (unpublished, 1960) has observed, in sera from a mother and her daughter, a complex series of haptoglobin zones, some of which do not migrate at the same rate as any of the previously described haptoglobins. This has been called the Johnson phenotype. Galatius-Jensen (1958b) has found in several members of a family a haptoglobin pattern in which components similar to those in both the 2-1 and 2-2 phenotypes were present together. Harris *et al.* (1959) have described a family in which there was an atypical segregation of the common haptoglobin types. There were several individuals with the 2-2 phenotype, who were children of mothers with the 1-1 phenotype, and it seemed likely that a rare modifying gene or uncommon allele was segregating in the family.

3. The Transferrins

The application of starch-gel electrophoresis to the study of human plasma led to the discovery of yet another series of genetically determined protein variants. The protein concerned here is transferrin, the iron-binding globulin which forms part of the β fraction in conventional electrophoresis. Before its identification as transferrin, this component in the starch-gel electrophoresis pattern was referred to as β-globulin C. So far, nine different kinds of transferrin have been identified. They have been called transferrins B_0, B_1, B_2, C, D_0, D_4, D_1, D_2, and D_3 (Smithies, 1957, 1958; Harris, Robson & Siniscalco, 1958; Giblett, Hickman & Smithies, 1959; Harris, Penington, Robson & Scriver, 1960) (see fig. 3). They differ one from another in their electrophoretic mobility, transferrin B_0 moving most rapidly towards the anode at pH 8.6, and the others following in the order given. Transferrin C occurs in most people, and the other variants are relatively uncommon. They have generally been found in combination with transferrin C, the unusual variant being present in amounts about equal to that of transferrin C. In such cases, about half the amount of transferrin C is present, compared with the amount generally found when it occurs alone.

The mobilities of the different transferrins relative one to another appear to be similar in conventional electrophoresis to those found in starch-gel electrophoresis, though the resolution is much sharper in the latter. Thus, differences in charge rather than in molecular size are probably the significant thing.

FIG. 3. Diagram of Starch-Gel Electrophoretic Pattern, Illustrating the Relative Mobilities of the Different Transferrin Variants (pH 8.6)



Family studies have so far been reported for the phenotypes: Tf B_1C (Harris *et al.* 1958), Tf B_2C (Smithies, 1958; Smithies & Hiller, 1959), Tf CD_1 (Horsfall & Smithies, 1958; Smithies & Hiller, 1959) and Tf CD_4 (Harris, Penington, Robson & Scriver, 1960). These studies suggest that there exists a series of genes, each of which determines the formation of a particular transferrin. Individuals with two of the above transferrins appear to be heterozygotes, individuals with one, homozygotes. Thus, individuals with Tf B_1C can be regarded as heterozygous for the gene determining Tf C and the gene determining Tf B_1, and so on. The only homozygous phenotype so far observed, apart from Tf C, has been Tf D_1.

There are quite marked variations in the frequencies of these various phenotypes in different human populations. Phenotype Tf B_2C, for example, occurs with an incidence of about one in 100 in European populations, but has not been reported among negroes. Phenotype Tf CD_1, on the other hand, has been found with a frequency as high as one in 10 in several negro populations, but has not been observed in Europeans. The other phenotypes all appear to have lower frequencies than these. So far no specific association between an unusual transferrin and any particular clinical disorder has been found.

4. Double Albumin

In 1957 Knedel reported finding two distinct albumin components in the serum of a single individual. Several relatives of the affected individual were also found to show the same phenomenon. Since then, a number of other families in which a similar peculiarity was segregating have been described (Nennstiel & Becht, 1957; Bennhold, Ott & Scheurlen, 1958; Earle, Hutt, Schmid & Gitlin, 1959; Wuhrmann, 1959; Franglen, Martin, Hargreaves, Smith & Williams, 1960).

The two albumin components could in each case be separated electrophoretically. The component moving more rapidly towards the anode pH 8.6 had the same mobility as the serum albumin in most normal individuals, and is probably identical with it. The slower-moving component probably represents a variant of normal serum albumin. It has been shown to behave immunochemically like normal serum albumin, and resembles it closely in other respects. About equal amounts of the two components were present in the sera of affected individuals, and the sum of them was about the same as the amount of albumin in normal serum. There seems to be no clinical abnormality consistently associated with the presence of the anomalous protein.

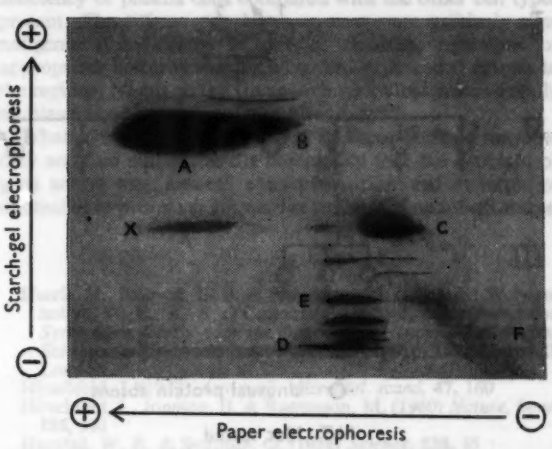
The family studies leave little doubt that the affected individuals are heterozygous for a rare gene. Presumably the normal gene at this locus is necessary for the formation of normal serum albumin, and its mutant form results in the formation of the albumin variant. The frequencies of the heterozygotes must be very low, so that the probability of encountering a homozygote with only one variant type of albumin in the serum is remote.

5. A New "Albumin-Like" Protein

In the course of studying the genetics of the haptoglobin and transferrin variants, Fraser, Harris & Robson (1959) encountered another form of genetically determined peculiarity of plasma protein synthesis, which differs in a number of respects from those described above. Several individuals, all members of the same family, were found to have a plasma protein component different in its electrophoretic properties from any of those usually observed in plasma when examined under the particular conditions then in use. The unusual protein could be detected by uni-dimensional starch-gel electrophoresis in which it moved slightly faster than transferrin C. In the uni-dimensional system it resembled in behaviour certain of the transferrin variants. However, in two-dimensional electrophoresis (Poulik & Smithies, 1958) it was found to behave quite differently (fig. 4). The protein migrated in the paper electrophoresis dimension at pH 8.6 with about the same mobility as serum albumin, and not in the β-globulin fraction, as does transferrin. Further evidence that it was not transferrin was provided by the demonstration that it did not bind radioactive iron. Thus, the unusual protein behaved like albumin in paper electrophoresis, but moved much more slowly in starch-gel electrophoresis. It was subsequently found that it reacts like albumin with a specific anti-albumin serum, and it also resembles albumin in certain of its other properties.

If much larger quantities of normal plasma than are necessary to demonstrate this unusual protein in affected individuals, are subjected to two-dimensional electrophoresis under the same conditions, a component with similar electrophoretic properties can often be detected in traces. Thus, it may be that the abnormality represents an increase in the quantity of the component rather than the formation of a qualitatively new protein. However, other interpretations are possible and, until the protein can be isolated in sufficient quantity to study its structure, its origin must remain uncertain.

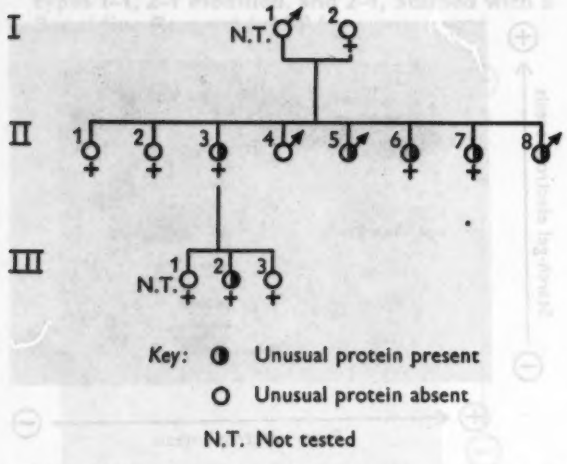
FIG. 4. Two-Dimensional Electrophoresis of Plasma Containing the Unusual "Albumin-Like" Protein



The paper electrophoresis dimension was carried out in an 0.05 M barbitone buffer of pH 8.6, and the starch-gel electrophoresis dimension in the discontinuous buffer system of Poulik (1957). A small amount of haemoglobin solution was added to saturate the haptoglobins.

A: albumin
B: α_1-globulin
C: β-globulin C (transferrin)
D: slow α_2-globulin
E: haptoglobin-haemoglobin complexes
F: γ-globulin
X: unusual component

FIG. 5. Pedigree of Family Showing the Segregation of the Unusual "Albumin-Like" Protein



The pedigree of the family in which the protein was observed is shown in fig. 5. It is consistent with the hypothesis that the individuals who formed the protein in significant amounts were heterozygous for a rare gene. The peculiarity did not appear to be specifically associated with the syndrome of goitre and deafness, which was also segregating in the family, or with any other obvious clinical peculiarity.

6. Discussion

The account given above has been confined to inherited variations in plasma proteins that can be detected electrophoretically. Many other plasma protein variations are known, however, which require other techniques for their demonstration. It must be remembered that marked changes in the structure and properties of a protein can occur without any significant change in its charge or size, and hence in its electrophoretic behaviour. Furthermore, many proteins in plasma occur only in extremely small amounts, and their variations are more readily susceptible to investigation by techniques taking advantage of their specific functional properties rather than by their electrophoretic behaviour. Typical examples are the serum cholinesterase variants, and the abnormality in caeruloplasmin formation occurring in Wilson's disease. One may also mention the various coagulation disorders such as haemophilia, Christmas disease, Owren's disease, and so on, each of which appears to have as its basis a deficiency or abnormality of one or another of a number of trace proteins necessary for the normal coagulation of blood.

Any discussion of these genetically determined peculiarities in plasma protein synthesis necessarily involves a consideration of how far they can be interpreted in terms of the hypothesis that each gene expresses its effects by specifying the amino-acid sequence in a particular protein or polypeptide chain. In particular the demonstration by Ingram (1957),[1] that haemoglobins A, S, C and other haemoglobin variants differ from one another simply in the substitution of a single amino-acid residue, naturally raises the question as to whether analogous differences form the basis of the plasma protein variations.

The situation which perhaps most clearly resembles the one exemplified by the haemoglobins is that provided by the series of transferrin variants. In both cases a number of genes occur in human populations, each of which results in the synthesis of a specific variant of a particular protein type. Heterozygotes, where two such genes are present, synthesize more than one distinct form of the protein. Although not much is yet known about the structural peculiarities of the transferrin variants, it seems plausible to suppose that, like the haemoglobins, they may differ from one another by relatively subtle modifications in their amino-acid sequences. The same is also likely to be true of the alternative form of albumin originally described by Knedel (1957). The haptoglobin situation may perhaps also be explained in the same terms, if it is assumed that the alternative polypeptide chains presumed to be determined by the various haptoglobin alleles can, in certain combinations, associate and polymerize to give the various complex phenotypes which are observed (Allison, 1959; Smithies & Connell, 1959).

Afibrinogenaemia and analbuminaemia present a different kind of problem. Here, the effect of the mutant gene in homozygotes is an almost complete failure in synthesis of a particular protein, and no alternative kind of protein is formed in its place. Several hypotheses can be considered. One possibility is that one is dealing with a chromosomal deletion. This seems unlikely, however, because the defect in synthesis is probably not quite complete, and minute amounts of protein are apparently formed. Another possibility is that gene mutation prescribes a new protein structure which is formed at a much slower rate, or is much more unstable, than its normal counterpart, and consequently is found in only minute amounts. In this case, the traces of fibrinogen or albumin present in the two conditions might be expected to be structurally different from their normal counterparts, even though they behave similarly immunologically. A third hypothesis might be that the protein deficiency may not be a direct consequence of a mutation of a gene which normally determines the structural organization of the particular protein, but may be a secondary consequence of the effects of a mutant gene concerned specifically in determining the structure of some other protein (perhaps an enzyme), which in turn is necessary for the synthesis of the protein whose deficiency was directly observed. The underlying defect may be more subtle and less easily detected than its more obvious secondary consequences.

In this connexion, it is worth mentioning that, although a particular gene may prescribe the structure of a particular protein and must therefore occur in an individual for that protein to be synthesized, other factors must be important also in deciding whether or not the synthesis of the protein should occur in a given cell at a certain time. Thus, albumin and fibrinogen are thought to be synthesized mainly, if not entirely, in liver cells in normal individuals. Yet cells in other tissues must also contain the genes which specify the amino-acid sequences of these proteins. The metabolic situation in liver cells presumably differs from that in other cells in that it provides the most suitable conditions for these proteins to be synthesized in normal individuals. In so far as this metabolic pattern is susceptible to alteration as a result of mutations of genes quite different from those prescribing the structure of

[1] See also Ingram, *Brit. med. Bull.* 1959, 15, 27.—Ed.

albumin or fibrinogen, the rate of synthesis of these two proteins might be markedly altered and, conceivably, almost completely suppressed.

Agammaglobulinaemia provides a similar, though probably even more complicated, problem. Here, not only is there a gross, though not complete, deficiency of the series of molecular species which comprise normal γ-globulin, but there has also been demonstrated a deficiency of two other immunochemically distinct protein components. The abnormality is reflected, too, at the cellular level. γ-Globulins are now generally believed to be synthesized by the plasma cells present in the lymph nodes, bone-marrow and other tissues. Good (1955) has shown that, both in the lymph nodes and bone-marrow in agammaglobulinaemia, there is a relative deficiency of plasma cells compared with the other cell types present. The difference is shown up even more strikingly after antigenic stimulation. In normal children, injections of appropriate antigens lead to a marked plasma-cell cytosis in the regional lymph nodes. In agammaglobulinaemia, virtually no plasma-cell response occurs (Good, 1955).

Whatever the final explanation of these findings may be, they serve to emphasize the complexity that the problem of gene action may assume, even when examined in terms of anomalies in protein synthesis due to single gene substitutions.

* * *

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AMINOACIDURIA

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1 Concentrations of amino acids in blood and urine
2 The genetically determined aminoacidurias
3 Discussion
References

Our knowledge of aminoaciduria is as old as our knowledge of the amino acids themselves; cystine, the first amino acid to be isolated, was prepared by Wollaston (1810) from a urinary calculus. During the following century and a half much more was learned about cystinuria, but the discovery of other abnormalities of amino-acid excretion had to await the development of suitable techniques for identifying or even detecting the presence of amino acids in the urine. Techniques for determining "amino-acid nitrogen" were developed by Folin (1922) and by Van Slyke, MacFadyen & Hamilton (1943); these determined the sum total of all the twenty or more different amino acids present, but were of little use in detecting or investigating the excretion of a single or a few amino acids, such as so often occurs in the genetically determined aminoacidurias.

The development of two-dimensional paper chromatography by Martin, Synge, and their co-workers (Gordon, Martin & Synge, 1943; Consden, Gordon & Martin, 1944) provided a simple technique by which each amino acid in a mixture could be identified and the amount present roughly estimated. The method was soon applied to the already known aminoacidurias, and led to new knowledge and greatly increased ease and certainty of diagnosis (Dent, 1946, 1949; Woolf, 1951a). Paper chromatography has played the dominant part in the discovery and investigation of all the abnormalities of amino-acid excretion discovered in recent years.

Other techniques that have proved useful in this field are column chromatography on ion-exchange resins (Moore & Stein, 1951; Moore, Spackman & Stein, 1958), high- and low-voltage electrophoresis (Michl, 1952) and microbiological assay of single amino acids (Yeh, Frankl, Dunn, Parker, Hughes & György, 1947).

1. Concentrations of Amino Acids in Blood and Urine

When proteins are eaten they are broken down to amino acids by enzymes in the gut. The amino acids are absorbed into the blood stream, then enter the cells of the body, often against a concentration gradient, and are built up into proteins. The body proteins are in dynamic equilibrium with the free amino acids in the blood; isotopic methods have shown that most, or all, of the proteins of the body are being continuously broken down to amino acids and continuously resynthesized, partly from their own breakdown products and partly from amino acids derived from the diet. In some proteins the change-over is rapid, e.g., fibrinogen with a physiological half-life of two to three days; in others it is too slow to be measured, e.g., collagen in adults.

Each amino acid is being constantly metabolized through a series of steps to, finally, urea, carbon dioxide and water (and sulphate in the case of the sulphur-containing amino acids). Each step involves a single enzyme catalysing a simple chemical change, and each step is obligatory. If the enzyme is absent or inactive, its substrate, whether a particular amino acid or its intermediate metabolite, usually accumulates or is excreted—only for a few amino acids are there efficient alternative metabolic pathways. This catabolism of amino acids takes place mainly in the liver and is the chief way in which the body rids itself of the various amino acids.

There is a loss of amino acids in the urine, which is quantitatively less important. The glomerular filtrate contains the free amino acids in the same concentrations as in the plasma, and as it passes through the proximal renal tubule each amino acid is reabsorbed. Groups of amino acids share a common site of reabsorption (e.g., lysine, ornithine, cystine and arginine). The reabsorption of the amino acids, like that of the sugars, is largely an active energy-requiring function of the cells of the proximal tubule. It is a function that can easily be disturbed by enzyme poisons, or, for example, by a deficiency of potassium, leading to a general aminoaciduria. For each amino acid there is a maximum rate at which it can be reabsorbed from the glomerular filtrate, though this is less sharply defined than in the case of glucose. The efficiency of reabsorption varies from one amino acid to another, so that their relative concentrations in normal urine are very different from those in plasma. Each free amino acid present in plasma can be detected in urine, though often in extremely low concentration.

2. The Genetically Determined Aminoacidurias

The application of the newer techniques to the study of blood and urine has shown that the genetically determined aminoacidurias can be divided into three main classes: (i) those lacking an enzyme necessary for the catabolism of an amino acid, thus leading to its accumulation in blood and tissues and so to its excessive excretion by an "overflow" mechanism; (ii) those in which, through some hereditary defect of the renal tubules, the ability to reabsorb a specific amino acid or a group of amino acids from the glomerular filtrate is diminished or absent; (iii) those in which the kidney is exposed to some toxic substance produced through an inborn error of metabolism, leading to progressive, but sometimes reversible, loss of reabsorptive ability by the renal tubule. A classification of the genetically determined aminoacidurias is given in Table I, and brief notes follow hereon.

Cystinuria. The excessive excretion of cystine, often leading to stone formation because of its insolubility, has been recognized for 150 years. The diamines, cadaverine and putrescine, were for long also believed to be excreted (von Udránszky & Baumann, 1889). However, Ackermann & Kutscher (1912) and Yeh *et al.* (1947) found lysine in the urine, and paper chromatography has shown that persons with cystinuria excrete cystine, lysine, arginine and sometimes ornithine (Dent & Rose, 1951). The diamines previously found are artifacts produced from the basic amino acids. The

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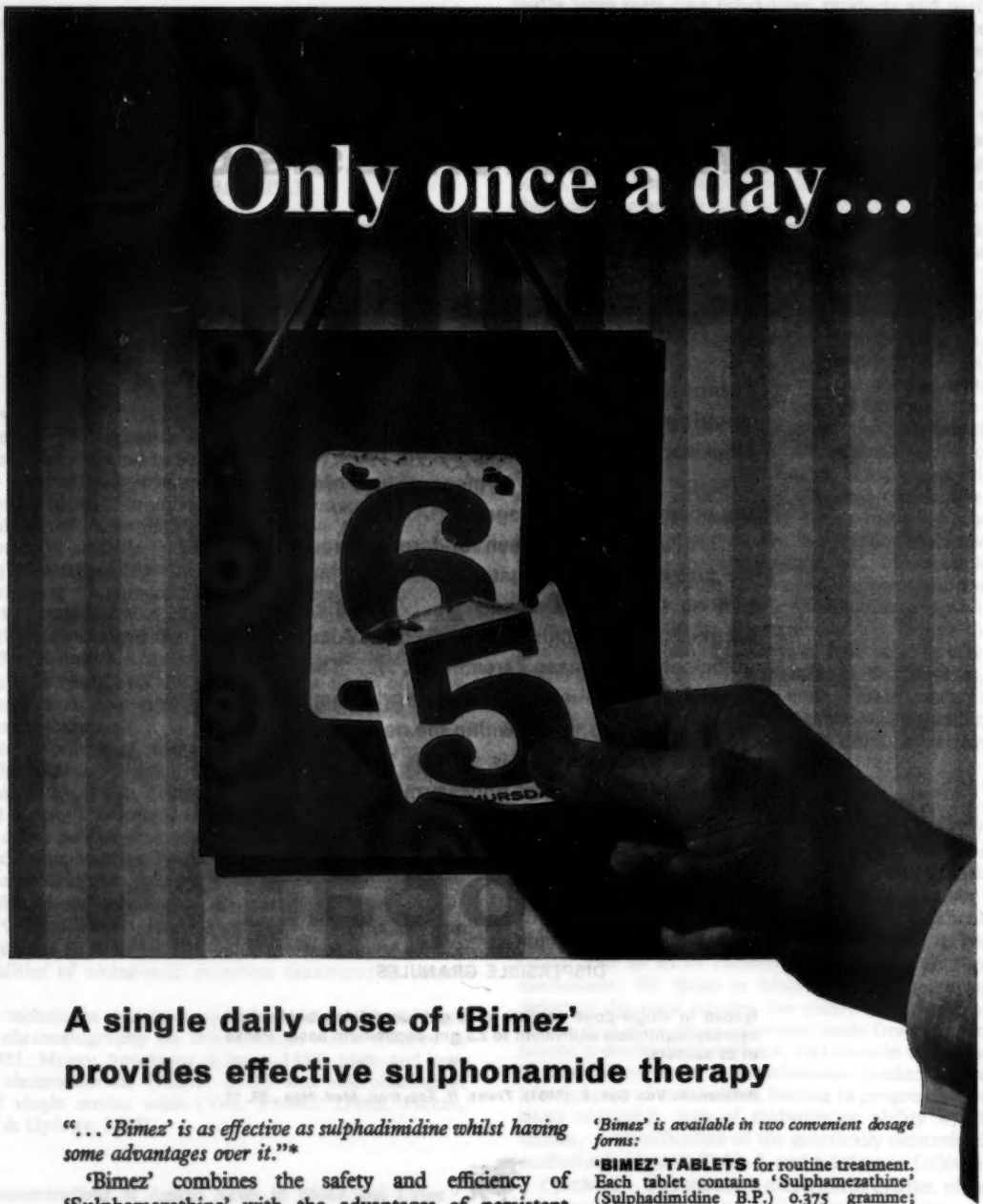

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TABLE I. Classification of the Genetically Determined Aminoacidurias

Condition	Amino acids excreted in urine	Other substances excreted in urine	Pathogenesis	Main clinical features	Heredity	Heterozygote expression
Cystinuria	Cystine; lysine; arginine; ornithine	—	Specific renal tubular defect	Urinary calculi	Recessive	*
Phenylketonuria	Phenylalanine	Phenylpyruvic acid and metabolites; indolyllactic and indolylacetic acids	Absence of phenylalanine hydroxylase	Mental defect	Recessive	†
Maple-syrup urine disease	Leucine; valine; isoleucine	α-Keto acids; α-hydroxy acids; indolyllactic and indolylacetic acids	Missing enzyme(s)	Cerebral degeneration	Probably recessive	‡
Hypophosphatasia	Phosphorylethanolamine	—	Lack of phosphatase	Bony changes	Recessive	*
Galactosaemia	As in plasma §	Galactose; protein	Absence of transferase	Liver damage; cataracts; mental defect	Recessive	†
Wilson's disease	? As in plasma §	Copper; sometimes glucose	Defect in copper metabolism	Cirrhosis; lenticular degeneration	Recessive	?†
Cystinosis	As in plasma §	Glucose; phosphate; K^+; HCO^-; protein	?	Dwarfing; rickets; acidosis; early death	Recessive	‡
β-Amino*iso*butyric aciduria	β-Amino*iso*butyric acid	—	Probably missing enzyme	Benign in character	Recessive	‡
Cystathioninuria	Cystathionine	—	Probably missing enzyme	? Mental defect	?	‡
Argininosuccinic aciduria	Argininosuccinic acid	—	?	Mental defect	?	‡
Hartnup syndrome	Tryptophan and many others	Indican; indolylacetic acid and its conjugates	Defects in amino-acid transport	Pellagra (variable)	Recessive	‡
Benign familial aminoaciduria	As in plasma §	Glucose; protein; sometimes phosphate	Renal tubular defect	Benign in character	Dominant	*
Glycinuria	Glycine	? Oxalate	Specific renal tubular defect	Urinary calculi	Dominant	*
Lowe-Terrey-MacLachlan syndrome	Lysine; arginine; histidine; tyrosine and many others	Organic acids; protein; glucose	Multiple congenital abnormalities	Cerebro-ocular malformations	?	‡
Infantile hepatic and renal dysfunction	As in plasma, but all in very high concentration; tyrosine prominent §	Glucose; phenolic acids; protein; phosphate; HCO_3^-	?	Jaundice; early death; rickets; acidosis; cirrhosis	?	‡
De Toni-Debré-Fanconi syndrome	As in plasma §	Glucose; phosphate; HCO^-	Variable	Rickets; acidosis	Variable	‡
Osteomalacia with aminoaciduria	As in plasma §	Glucose; phosphate	Renal tubular defects	Osteomalacia	Recessive	‡

* Some or all heterozygotes spontaneously show aminoaciduria.
† Heterozygotes show abnormality on a loading test.
‡ No heterozygote expression detected.
§ All the amino acids present in plasma appear in the urine in approximately the same concentrations, relative one to another, as in plasma.

blood concentrations of cystine, lysine and arginine are normal or low in cystinuria, proving that there is a renal tubular failure to reabsorb these amino acids. Intravenous infusion of lysine causes excessive excretion of cystine and arginine as well as lysine (Robson & Rose, 1957), indicating competition between these amino acids for a single tubular absorption site.

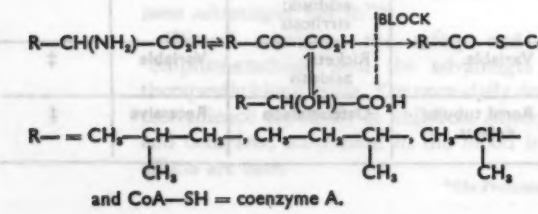
Family studies show that the condition is carried by a single recessive gene (Harris, Mittwoch, Robson & Warren, 1955). In about a third of heterozygotes the level of excretion of cystine or lysine is intermediate between the normal and the cystinuric levels, though arginine and ornithine excretion are normal; in the remaining two-thirds, the urinary amino acids are normal.

If cystinuric subjects escape stone formation they have normal health. The most effective treatment, apart from surgical removal of stones, is giving enough water to ensure that the urine is dilute at all times, so hindering the crystallization of cystine.

Phenylketonuria. Phenylketonuria was identified by Fölling (1934). In normal individuals, phenylalanine is converted to tyrosine, as the obligatory first step in its catabolism, by phenylalanine hydroxylase of liver. Persons with phenylketonuria lack this enzyme, and, in consequence, phenylalanine accumulates in blood, the cerebrospinal fluid, and the tissues, and spills over into the urine. Some of the phenylalanine is converted to phenylpyruvic acid (Fölling, 1934), phenyllactic acid (Jervis, Block, Bolling & Kanze, 1940), phenylacetylglutamine (Woolf, 1951b) and *o*-hydroxyphenylacetic acid (Armstrong, Shaw & Robinson, 1955), all of which appear in the urine. Almost invariably there is very severe mental deficiency and often evidence of neurological damage.

Treatment consists of giving a diet low in phenylalanine (Woolf & Vulliamy, 1951). If so fed from early infancy the child develops normally; if the diet is started later on, there is often some improvement in the phenylketonuria (Armstrong, Low & Bosma, 1957; Woolf, Griffiths, Moncrieff, Coates & Dillistone, 1958; Brimblecombe, Stoneman & Maliphant, 1959; Murphy, 1959).

Maple-syrup urine disease. This is a familial severe cerebral degeneration, usually leading to death in infancy, and was first described by Menkes, Hurst & Craig (1954). The high levels of leucine, isoleucine and valine in the blood and urine were recognized by Westall, Dancis & Miller (1957). Three groups of workers (Dancis, Levitz, Miller & Westall, 1959; MacKenzie & Woolf, 1959; Menkes, 1959), independently and almost simultaneously suggested that there was a metabolic block in oxidative decarboxylation of the branched chain α-keto acids, i.e., in the second step in the metabolic degradation of leucine, isoleucine and valine.



In consequence the α-keto acids accumulate; they are partially converted to the α-hydroxy acids, and partially transaminated back to the amino acids. All nine compounds accumulate in the blood and overflow into the urine.

Very few cases have been described. The condition is probably inherited recessively (Lane, 1961), but the absence of consanguinity among the parents of known subjects suggests that the gene is not very rare. The disease may occur as frequently as phenylketonuria, but be missed because it is usually fatal during the first few weeks of life, and cases may not be differentiated from general perinatal mortality.

Treatment with a diet low in leucine, valine and isoleucine is effective in preventing cerebral degeneration (Holt, Snyderman, Dancis & Norton, 1960).

Hypophosphatasia. This defect in bone formation is associated with a very low serum alkaline phosphatase level (Rathbun, 1948; McCance, Fairweather, Barrett & Morrison, 1956). Phosphorylethanolamine (β-aminoethylphosphoric ester) is found in large amounts in the urine (Fraser, Yendt & Christie, 1955; McCance, Morrison & Dent, 1955). The presumed heterozygotes often excrete smaller, but still abnormal, amounts of this amino acid (Harris & Robson, 1959).

Galactosaemia. Absence of the enzyme galactose 1-phosphate uridyl transferase causes large amounts of galactose and galactose 1-phosphate to accumulate in blood and tissues. These are toxic, probably through inhibition of phosphoglucose mutase by the galactose 1-phosphate, and, among other effects, they cause the renal tubular cells to lose their power to reabsorb any amino acids from the glomerular filtrate. Thus there is a generalized aminoaciduria.[1]

Withdrawal of galactose from the diet results in completely normal mental and physical development. The gross aminoaciduria clears up most dramatically and completely, usually within three days. However, the urinary amino-acid pattern does not always return to normal; instead, there is sometimes revealed a secondary aminoaciduria, with a moderately high excretion of ethanolamine and β-amino*iso*butyric acid, presumably owing to the liver dysfunction known to be associated with the disease, which persists for some months.

Wilson's disease. In this, an abnormality of copper metabolism is involved (Rumpel, 1913; Glazebrook, 1945; Cumings, 1948), and, at autopsy, the copper content of a number of organs, especially brain, liver and kidney, is found to be high.[2] In the blood of most patients with Wilson's disease, caeruloplasmin is present in low concentration or absent (Cartwright, Hodges, Gubler, Mahoney, Daum, Wintrobe & Bean, 1954; Bearn & Kunkel, 1955), or has an abnormally low turnover rate (Rosenoer & Franglen, 1959; Sass-Kortsak, Cherniak, Geiger & Slater, 1959). The primary site of gene action has still to be determined.

Aminoaciduria very frequently occurs in Wilson's disease and is sometimes accompanied by glucosuria (Uzman & Denny-Brown, 1948; Cooper, Eckhardt, Faloon & Davidson, 1950). The blood amino-acid levels are normal; it follows that, in spite of the hepatic cirrhosis, this is a renal and not an overflow aminoaciduria. It seems probable that copper is deposited in the cells of the renal tubules and, like other heavy metals, poisons the enzyme systems necessary for the reabsorptive functions of the cells. It is possible that a study of cirrhosis in children will reveal a more wide-spread incidence of the biochemical features of Wilson's disease, without gross neurological signs.

[1] See also Holzel, p. 213 of this number of the Bulletin.—Ed.
[2] See also Walshe, *Brit. med. Bull.* 1957, 13, 132.—Ed.

Cystinosis (*cystine storage disease*; *Lignac-Fanconi disease*). Cystine storage was first described by Abderhalden (1903). The clinical features, biochemical findings, genetics, and morbid anatomy of the condition have been described by Lignac (1924), Fanconi (1936), and Bickel *et al.* (1952).

The first signs of disease are usually a failure to thrive, accompanied by polyuria and polydipsia. Onset of symptoms usually occurs at five to nine months of age, but can be much later. The urine is very dilute, and contains protein (about 50–100 mg./100 ml.) and glucose (50 mg./100 ml. or more). Amino acids are present in the urine in the same concentrations, relative one to another, as in the blood; this constitutes a "renal" chromatographic pattern. The concentrations of certain amino acids in the blood are raised to about twice the normal level (Woolf, 1951a; Bickel, 1955; Brigham, Stein & Moore, 1960); none the less, these concentrations remain well below the normal renal threshold, and the aminoaciduria is clearly due to a renal leak. Potassium, bicarbonate and phosphate are also lost in the urine, and this causes the most serious effects of the disease—hypokalaemia, acidosis and hypophosphataemic rickets. The most prominent clinical feature is marked dwarfing. The deposition of crystals of cystine in the reticulo-endothelial system is characteristic, and their detection in the cornea (by slit lamp), in an excised lymph node (alcohol-fixed), or in bone-marrow is diagnostic.

Most of the clinical and biochemical features can be explained by progressive loss of function of the proximal and distal renal tubules. Clay, Darmady & Hawkins (1953) detected a characteristic "swan-neck" deformation[*] of the tubules, a feature Darmady also finds in some other diseases (Giles, Pugh, Darmady, Stranack & Woolf, 1957). The storage of cystine in the reticulo-endothelial system and the raised blood concentrations of some amino acids indicate a more deep-seated error of metabolism—at present unknown—as the primary defect.

This disease is carried by a single recessive gene, and no heterozygotic effect has been detected. Death usually occurs in childhood, but, if large doses of potassium, citrate and phosphate are given, life can be prolonged. Cystinosis is a clear-cut clinical, biochemical and genetic entity. However, it is often lumped together with other conditions, showing some of its features, under the term "Fanconi syndrome".

β-*Aminoisobutyric aciduria*. This was first described by Crumpler, Dent, Harris & Westall (1951). Harris (1953) showed it was familial with a recessive mode of inheritance. The disease is probably due to deficiency of an enzyme for further metabolism of this β-amino acid, an intermediate in the breakdown of thymine (Fink, 1956; Gartler, 1959). β-Amino*iso*butyric acid is also excreted by patients with chronic myeloid leukaemia (Gartler, Firschein & Gidaspow, 1957), by some patients with liver disease (Woolf, 1951a) or the nephrotic syndrome (Woolf & Giles, 1956), and by many very young infants (Woolf & Norman, 1957).

Cystathioninuria. Excretion of cystathionine was found by Harris (1959) in a mentally retarded adult and in normal relatives. It appears to arise through a block in the metabolism of methionine.

Argininosuccinic aciduria. The excretion of argininosuccinic acid (an intermediate in the synthesis of urea) by two mentally retarded sibs was described by Allan, Cusworth, Dent & Wilson (1958) (see Westall, 1960). Argininosuccinic acid occurs, in these cases, in high concentration in the c.s.f., low concentration in the blood, and fairly high concentration in the urine.

Hartnup syndrome. Baron, Dent, Harris, Hart & Jepson (1956) described three sibs suffering from a pellagra-like syndrome with cerebellar ataxia, mental deficiency, personality defects, a photo-sensitive dermatitis, and a marked aminoaciduria and indoluria. Tryptophan was the most prominent amino acid, and the other more prominent indole derivatives present in the urine were indican, indolylacetic acid, indolylacetyl-glutamine and indolylacetylglucuronide (Jepson, 1956). Family studies indicate a recessive mode of inheritance, and a number of cases previously considered to be pellagra have now been rediagnosed as cases of the Hartnup syndrome. The basic defect appears to be a failure in amino-acid absorption, both from the gut and from the glomerular filtrate (Milne, Crawford, Girão & Loughridge, 1960). In consequence, amino acids remain in the gut far longer than normal and undergo bacterial attack, tryptophan being partly converted to indole and indolylacetic acid, which are absorbed, conjugated and excreted in the urine. There is also a renal loss of amino acids. There may also be defective transport across the cell membranes. Large doses of nicotinamide are beneficial and some individuals with the biochemical lesion are free from mental or neurological signs of disease (Hersov & Rodnight, 1960).

Benign familial aminoaciduria. Luder & Sheldon (1955) described a pair of identical twin girls who, with their father, had a renal glucosuria and a very marked general renal aminoaciduria. All three had a small amount of serum protein in the urine (about 50 mg./100 ml.) but it was well concentrated (cf. section on cystinosis) and there was no evidence of renal acidosis. The fasting plasma amino-acid concentrations were all well below normal, though the distribution was normal. The paternal grandfather may have had the same condition. One of the twins was much smaller than her sister, who was of normal stature, and the shorter twin later developed phosphate-losing rickets. This is evidently a dominantly inherited renal tubular defect affecting the reabsorption of glucose, all the amino acids, protein and, in one case, phosphate. It appears to be a harmless anomaly except where phosphate loss causes rickets and, perhaps, dwarfing. The incidence is unknown.

Glycinuria. A patient with recurrent bilateral nephrolithiasis was found to excrete large amounts of glycine by de Vries, Kochwa, Lazebnik, Frank & Djaldetti (1957). The stones were mainly composed of calcium oxalate and contained about 0.5% of glycine. The patient's sister also had renal stones and glycinuria, their mother glycinuria but no history of stones, and their maternal grandmother had glycinuria and a history of renal colic. Glycine excretion was about six times the normal, but all the other urinary amino acids were normal. Urinary oxalate, glucose, and phosphate were also normal. Glycinuria is evidently inherited in dominant fashion; it is probably coincidence that all affected members were female. Its relationship to nephrolithiasis is not clear.

Lowe-Terrey-MacLachlan syndrome. This was described as congenital mental retardation, hydrophthalmos (glaucoma), decreased renal ammonia production and organic aciduria (aminoaciduria) (Lowe, Terrey & MacLachlan, 1952). In some cases there were cataracts (Jagenburg, 1959) and brain malformations. Only boys are affected.

Congenital hepatic and renal dysfunction. Occasionally,

[*] See also Darmady & Stranack, *Brit. med. Bull.* 1957, 13, 21.—ED.

infants are seen who develop deep jaundice soon after birth and have a very marked glucosuria, massive aminoaciduria, some proteinuria, and very high excretion of *p*-hydroxyphenylpyruvic and other phenolic acids (Baber, 1956). The presence of glucose rather than galactose in the urine is the easiest and quickest way of distinguishing the condition from galactosaemia. The aminoaciduria is of both "overflow" and "renal" type, and is more massive than that met in many other conditions; tyrosine is particularly prominent. The children usually die in the first few weeks of life, but some appear to survive and develop cirrhosis of the liver in later childhood. Their liver metabolism and renal tubular function sometimes become normal, and, in other cases, remain abnormal with glucosuria, gross aminoaciduria, and phenolic aciduria, and with acidosis and rickets tending to occur as a consequence of renal loss of bicarbonate and phosphate. There is some evidence that the condition is familial, but nothing is known of its genetics.

De Toni-Debré-Fanconi syndromes. These are a group of diseases in children showing acidosis, vitamin-resistant rickets, and renal loss of amino acids, glucose, phosphate, bicarbonate and potassium (Fanconi, 1931; de Toni, 1933; Debré, Marie, Cléret & Messimy, 1934). They are sometimes confused with cystinosis, and can be caused by renal glycogen storage (Fanconi & Bickel, 1949) or by nephrosis (Hooft & Vermassen, 1960), but in many cases there seems to be a primary renal tubular defect.

Osteomalacia with aminoaciduria. This is a disease of adults aged about 40 and is termed the "Fanconi syndrome" by Dent (Dent, 1947; Dent & Harris, 1956). There is a primary renal tubular defect leading to loss of phosphate, glucose and amino acids. It is recessively inherited and the aminoaciduria is present before the onset of symptoms.

3. Discussion

The way in which the gene acts is fairly clear in a condition such as phenylketonuria. Here the enzyme phenylalanine hydroxylase is evidently synthesized by the normal gene through very few intermediate steps. The mutant gene probably synthesizes some other protein which lacks enzymic properties. The mental, neurological, and other signs of phenylketonuria are plainly due to the toxic effect of the substances that accumulate as a direct effect of the absence of the enzyme. In those conditions involving a defect of renal tubular reabsorption, e.g., cystinuria, one can postulate the existence in the cell wall of an enzyme-like substance whose synthesis is under direct genetic control, and which selectively binds a specific amino acid or group of amino acids present in the glomerular filtrate. Our ignorance of the normal mechanism for renal reabsorption of amino acids makes it impossible to be very precise and, in any case, some additional explanation is necessary for those conditions inherited in a dominant fashion. In other conditions, e.g., cystinosis, we know still less about the causal relationship between abnormal gene and aminoaciduria.

Each of the conditions outlined above is notable for its rarity; in several cases the disease has been found in only a single family. This is not surprising when one considers that many of the diseases are lethal in childhood and nearly all are more or less disabling. A gene carrying such a character must be very rare unless there is some compensating advantage, and there is no evidence for this in these conditions. However, nearly every person known to have one or other of these conditions was investigated in the first place because he or she exhibited the symptoms of some disease or was related to someone known to have the condition. There have been very few studies of the incidence of these conditions in normal populations—two exceptions are the study of β-amino*iso*-butyric aciduria for ethnological ends (Gartler, Firschein & Kraus, 1957) and the testing of new-born infants for phenylketonuria (Gibbs & Woolf, 1959). The greatest difficulty is the labour of running chromatograms on a sufficient number of urine specimens to enable one to detect conditions occurring with a frequency of 1 in 10^4–10^6. One must select the material to be investigated, generally on clinical grounds. However, this in turn introduces uncertainties; for example, in the only known family with glycinuria the propositus presented with nephrolithiasis—but it is not known whether or not this was purely coincidental and whether glycinuria is a harmless anomaly occurring not infrequently. The accidental discovery, in the course of other investigations, of persons with phenylketonuria who are mentally completely normal, leads one to doubt whether, even in this much-studied disease, our knowledge is as nearly complete as we tend to assume.

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JOURNAL OF PATHOLOGY AND BACTERIOLOGY

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Volume LXXXII July 1961 No. 1

WITZLEBEN, C. L., and JOHN P. WYATT
The effect of long survival on the pathology of thalassæmia major (Plates I–III)
FLOREY, H. W., and L. H. GRANT
Leucocyte migration from small blood vessels stimulated with ultraviolet light: an electron-microscope study (Plates IV–XXIV)
AIDAROS, S. M., and LILY A. M. SOLIMAN
Portal vascular changes in human bilharzial cirrhosis (Plates XX and XXI)
WILLIS, J.
The induction of malignant neoplasms in the thyroid gland of the ra (Plate XXV)
SCLARE, G., and G. TAYLOR
Experimental thyroiditis and the placental transfer of auto-antibodies (Plates XXVI–XXVIII)
BECK, A.
Serological investigations of atypical acid-fast bacilli
SMITH, H. WILLIAMS, and W. E. CRABB
The fæcal bacterial flora of animals and man: its development in the young
DOUGLAS, S. W., and A. C. PALMER
Idiopathic demyelination of brain-stem and cord in a miniature poodle puppy (Plates XXIX and XXX)
HALLEY, J. B. W.
Epidermoid cyst of the testicle (Plate XXXI)
GIBSON, J. B.
The liver surface in the ascites of heart failure (Plate XXXII)
HOU, C. T. (HOU CHIEN-TSÜN)
Repair of the extrahepatic bile ducts after mechanical and chemical injury (Plates XXXIII–XXXV)
CAMERON, G. R., and C. T. HOU (HOU CHIEN-TSÜN)
The response of the intrahepatic bile-ducts to chemical injury (Plates XXXVI–XXXIX)
GRIFFITHS, D. B., K. R. REES and K. P. SINHA
Blood and bile composition in experimental biliary cirrhosis
WHEATER, D. W. F., and E. W. HURST
The effect of sex on bacterial infections in mice and on the chemotherapy of one of them
ADAMS, C. W. M., and N. A. TUQAN
Elastic degeneration as source of lipids in the early lesions of atherosclerosis (Plate XL)
POWELL, D. E. B.
Non-suppurative lesions in staphylococcal septicæmia (Plates XLI–XLIV)
SPRIGGS, A. I., and G. A. MEEK
Surface specialisations of free tumour cells in effusions (Plates XLV–L)
SPENCER, H.
Pulmonary blastomas (Plates LI–LIV)
MEEK, E. S.
The cellular distribution of deoxyribonucleic acid in primary and secondary growths of human breast cancer
BARRIE, H. J.
Herniations into the renal veins with special reference to hydronephrosis (Plates LV–LVII)
O'NEAL, ROBERT M., W. J. S. STILL and W. STANLEY HARTROFT
Experimental atherosclerosis in the rat (Plates LVIII–LXI)
MORRISON, R. B.
The effect of temperature and chloramphenicol on the development of flagella and motility in a strain of *Escherichia coli* (Plate LXII)

SHORT ARTICLES—
HAYES, J. A., and OWEN E. CHRISTENSEN, Primary adrenal lymphoma (Plate LXIII)
HIRSCH, H. A., and T. F. PAINE, Jr., Experimental uterine tetanus in mice
MALHOTRA, M. S., and H. C. WRIGHT, The effects of a raised intrapulmonary pressure on the lungs of fresh unchilled cadavers (Plates LXIV and LXV)
AINSWORTH, R. W., and G. A. GRESHAM, Giant-cell aortitis with rupture of the aorta (Plate LXVI)
PHILLIPS, J. E., The commensal role of *Actinobacillus lignieresi*
FUZI, M., and R. CSOKA, An egg-yolk reaction test for the differentiation of leptospiræ
CLAUSEN, O. G., Mutation of a beta-haemolytic streptococcus strain of group O
HUTCHISON, J. G. P., Crystals in colonies of *Staphylococcus aureus* (Plate LXVII)
ALEXANDER, M. K., A mixed tumour of the liver in an adult (Plate LXVIII)
CURRAN, R. C., and A. E. CLARK, A reliable autoradiographic technique

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VOL. VII, No. 3 CONTENTS *JULY 1961*

Differential Haemopoietic Response of Rat and Guinea Pig to Extracts of Human Urine. By D. G. OSMOND, P. J. ROYLANCE, J. M. YOFFEY and D. C. VAN DYKE.

Plasma Erythropoietin Levels in Anaemia: Studies Using an Assay Method in Intact Rats. By RICHARD W. PAYNE.

The Attachment of Proteins to Aldehyde-tanned Cells. By N. R. LING.

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An Investigation of the Haemorrhagic Tendency in Renal Failure. By M. L. N. WILLOUGHBY and SHEILA J. CROUCH.

Red-cell Metabolism in Non-spherocytic Congenital Haemolytic Anaemia. By MARGARET A. ROBINSON, P. BRONWEN LODER and G. C. DE GRUCHY.

Haemophilia Complicated by an Acquired Circulating Anticoagulant: A Report of Three Cases. By MICHAEL HALL.

The Preparation and Assay of a Christmas-factor (Factor IX) Concentrate and its use in the Treatment of Two Patients. By ROSEMARY BIGGS, ETHEL BIDWELL, D. A. HANDLEY, R. G. MACFARLANE, J. TRUETA, A. ELLIOT-SMITH, G. W. R. DIKE and B. J. ASH.

The Use of Factor VIII Freed from Fibrinogen in the Treatment of Haemophilic Patients. By A. PAVLOVSKY, C. SIMONETTI, G. CASILLAS, L. J. BERGNA, A. ANDINO and A. E. BACHMANN.

Hereditary Persistence of Foetal Haemoglobin: A Family Study Suggesting Allelism of the F Genes to the S and C Haemoglobin Genes. By J. E. MACIVER, L. N. WENT and R. A. IRVINE.

Erythrocyte Survival Time Determinations with the Aid of $DF^{32}P$. By J. G. EERNISSE and J. J. VAN ROOD.

Meeting of the British Society for Haematology: Summaries of Communications.

Book Reviews. Books and Monographs received.

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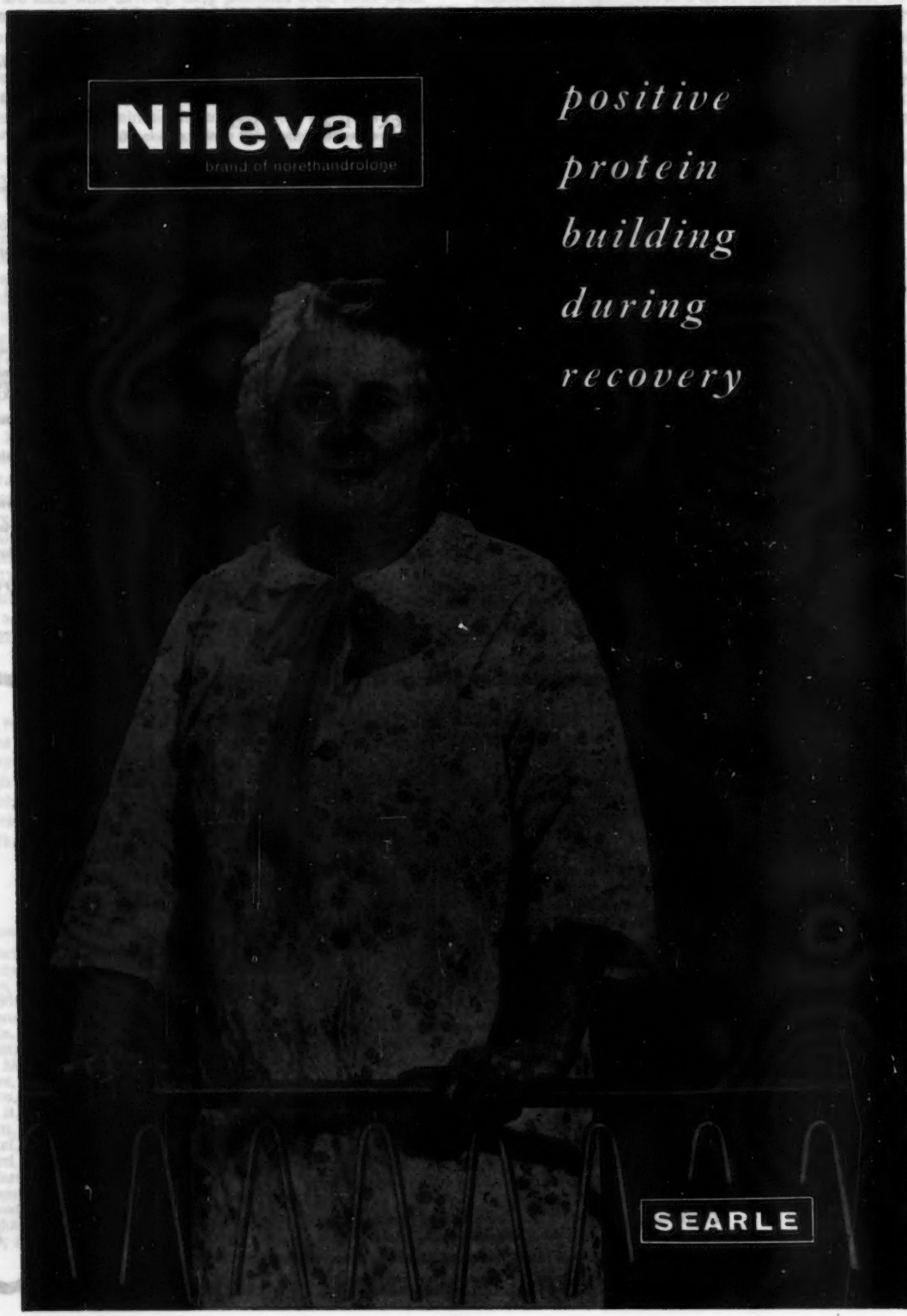
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PSEUDO-CHOLINESTERASE

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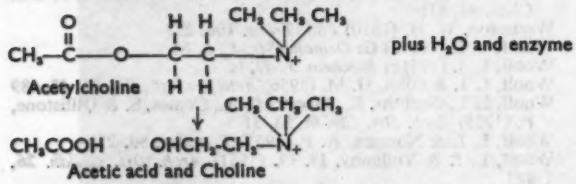
1 Cholinesterases
2 Suxamethonium
3 Inheritance of low plasma pseudo-cholinesterase activity
4 Atypical pseudo-cholinesterase
5 Population surveys
6 Mode of inheritance
7 Conclusion
References

1. Cholinesterases

Cholinesterases are enzymes which hydrolyse choline esters, producing free choline and the corresponding acid:



These enzymes are classified, on the basis of their physiological roles and biochemical behaviour, into two main types: true and pseudo-cholinesterase. This terminology was introduced by the late Professor B. Mendel who was largely responsible for the differentiation of the two groups (Mendel & Rudney, 1943).

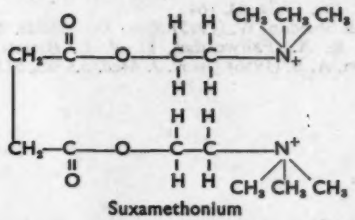
True cholinesterase, which is found mainly in nerve and muscle tissue and in red cells, is thought to be responsible for the destruction of acetylcholine at the neuromuscular junction. Biochemically, it is characterized by the rapid rate at which it hydrolyses acetylcholine, although it can split some other choline esters more slowly. Pseudo-cholinesterase is found in many tissues, but especially in glia, heart muscle, intestines, skin, liver and plasma. In human blood the two enzymes are sharply localized: true cholinesterase is found in the red cell and pseudo-cholinesterase in the plasma. However, this is not true of all species. Pseudo-cholinesterase can act on a wide variety of choline esters and can also hydrolyse acetylcholine, although relatively slowly. There are slight differences in the biochemical behaviour of the enzyme from various tissues and species, so that the term "pseudo-cholinesterase" refers to a family of enzymes. Under normal conditions complete inhibition of the plasma enzyme does not produce clinical symptoms. Indeed the function of the enzyme is unknown, although several theories have been proposed. We consider the most plausible is that it can protect against choline esters formed during metabolism (Lehmann & Silk, 1953). For example, propionylcholine, which has been isolated from ox spleen (Banister, Whittaker & Wijesundera, 1951), inhibits true cholinesterase and is broken down by pseudo-cholinesterase.

Pseudo-cholinesterase can be estimated by a method in which hydrolysis of acetylcholine in a bicarbonate buffer produces carbon dioxide, which is then measured manometrically. The micromanometer used is the Warburg apparatus. One unit of pseudo-cholinesterase is defined as that amount of enzyme which produces the evolution of one microlitre of carbon dioxide in one minute at 37° C. (McArdle, 1940). The normal range is 55–125 units/ml. The plasma enzyme is formed in the liver, and its level is reduced by liver cell damage. The estimation of plasma pseudo-cholinesterase has therefore proved a relatively sensitive test of liver function. Plasma levels may also be markedly depressed in malnutrition, cachexia, and in poisoning by organo-phosphorus compounds, which include the "nerve gases" and many modern insecticides.

2. Suxamethonium

Apart from its use in liver-function tests, pseudo-cholinesterase was of little clinical importance until 1949. In that year Bovet and his colleagues reported that suxamethonium was a potent muscle relaxant, while Bovet-Nitti showed that it was rapidly hydrolysed by pseudo-cholinesterase (Bovet, Bovet-Nitti, Guarino, Longo & Marotta, 1949; Bovet-Nitti, 1949). This enzymic inactivation is responsible for the brief action of suxamethonium which has proved so useful in anaesthesia. Other muscle relaxants are largely removed by excretion in the urine and therefore have a more prolonged effect.

Suxamethonium was first called "succinyldicholine" and it can also be regarded as the combination of two molecules of



acetylcholine. Pseudo-cholinesterase splits off the two choline groups in succession so that succinylmonocholine is formed as an intermediate in the hydrolysis (Whittaker & Wijesundera, 1952; Lehmann & Silk, 1953). Because of this rapid hydrolysis, suxamethonium produces a respiratory paralysis lasting only a few minutes. But patients with a plasma pseudo-cholinesterase level below 25 units/ml. can suffer a prolonged apnoea which may last for several hours. This is, of course, a rare event. In the early reports of these cases, the low enzyme levels were ascribed to liver disease or to other pathological causes (Bourne, Collier & Somers, 1952; Evans, Gray, Lehmann & Silk, 1952). But Davies (personal communication, 1951) found that the plasma pseudo-cholinesterase was virtually absent in an apparently healthy soldier, while both Bourne (1953) and Forbat, Lehmann & Silk (1953) described healthy young adults with a low pseudo-cholinesterase level discovered because of unexpected sensitivity to suxamethonium. Many similar cases have since been reported. Enzyme levels may be investigated in patients with liver disease before suxamethonium is injected but, at least in the past, levels of

pseudo-cholinesterase were not tested in healthy subjects. Consequently these rare healthy people with a low pseudo-cholinesterase level form the greater proportion of the suxamethonium-sensitive subjects now being discovered. For example, Lehmann & Ryan (1956) could not find a pathological cause for the low levels in 13 of 21 such patients. Most subjects with the inherited anomaly are discovered by their abnormal reaction to exhibition of suxamethonium.

3. Inheritance of Low Plasma Pseudo-Cholinesterase Activity

The low plasma pseudo-cholinesterase level occasionally seen in healthy subjects is now known to be inherited. Forbat *et al.* (1953) found four of these individuals, in fairly rapid succession, and in one instance they were able to obtain blood from a sibling. Here, also, a low pseudo-cholinesterase activity was found in association with perfect health. This strongly suggested that the abnormality was familial. By 1956, Lehmann and Ryan had investigated the families of five suxamethonium-sensitive propositi. In four families very low enzyme levels were discovered in siblings, nephews or grandchildren. The relatives of the fifth propositus appeared at that time to be normal. On the basis of these results, Lehmann and Ryan tentatively concluded that a recessive gene was responsible. Allott & Thompson (1956), who described one further family, suggested that the gene was not purely recessive, and that heterozygotes could be detected in whom the enzyme level was only slightly depressed.

This hypothesis was difficult to evaluate. There is a wide normal range of pseudo-cholinesterase values, and any heterozygotes are therefore difficult to differentiate. By 1958, Lehmann, Patston & Ryan had examined 72 relatives of 14 propositi and were able to support Allott and Thompson's suggestion. There were enzyme levels characteristic of each of the three genotypes, although with a considerable overlap between the normal homozygote and the heterozygote. Kaufman, Lehmann & Silk (1960) analysed the results from 26 families and suggested the following classification according to the enzyme level:—less than 26 units/ml.: abnormal homozygote; between 26 and 35 units/ml.: abnormal homozygote and heterozygote; between 36 and 60 units/ml.: heterozygote; between 61 and 90 units/ml.: normal homozygote and heterozygote; greater than 90 units/ml.: normal homozygote.

Using these figures, the genotypes can be identified with a reasonable degree of certainty in many families, but not in all. For instance, in one family described by Lehmann & Ryan (1956), the propositus had an enzyme level of 35 units/ml. and could, therefore, be either an abnormal homozygote or a heterozygote. If she was homozygous, both parents must have been heterozygotes, while if she was heterozygous, one parent could be a normal homozygote. The enzyme levels of the parents, 66 and 76 units/ml., respectively, did not help in choosing the correct alternative. Furthermore, there is some indirect evidence which suggests that the classification is not entirely accurate. The Mendelian theory predicts that, of the siblings of a homozygous propositus, 25% should be abnormal homozygotes, 25% normal homozygotes and 50% heterozygotes. In 18 families such siblings could be investigated. Thirteen of the 31 (i.e., 42%) had pseudo-cholinesterase levels of less than 26 units/ml. This suggests that a proportion of those classified as abnormal homozygotes are actually heterozygotes, unless there was an enormous positive natural selection for abnormal homozygotes.

4. Atypical Pseudo-Cholinesterase

Kalow and his colleagues, working in Canada, compared the pseudo-cholinesterases from normal and abnormal homozygotes by a spectrophotometric technique which enabled them to measure the activity of the enzyme with a variety of substrates and inhibitors (Kalow & Lindsay, 1955). They found that the pseudo-cholinesterase from the abnormal homozygote differed from the normal in two respects: it was less active against all substrates, and more resistant to most inhibitors (Kalow & Davies, 1958; Davies, Marton & Kalow, 1960). The detailed biochemical evidence showed that the two pseudo-cholinesterases were different enzymes. In the abnormal homozygotes, therefore, the usual pseudo-cholinesterase is not merely depressed but is replaced by another enzyme.

The local anaesthetic dibucaine (cinchocaine) is one of the inhibitors which is less effective against the atypical enzyme. Kalow & Genest (1957) used this to devise a test system which clearly differentiates sera from the two homozygotes: a given concentration of dibucaine inhibits the normal pseudo-cholinesterase by about 80%, and the atypical enzyme by only 20%. The degree of inhibition produced in this test is called the "dibucaine number".

The dibucaine number is independent of the enzyme concentration and is about 80% in the normal homozygote, even if disease has reduced the enzyme activity to that of the abnormal homozygote. This, incidentally, is of practical importance when a low pseudo-cholinesterase level is found and the problem arises of whether it indicates a decreased liver function. When sera from heterozygotes are examined the percentage inhibition by dibucaine is between 52% and 69%. In contrast to values obtained by mere estimation of enzyme activity, these values do not overlap with those found in either homozygote, and the heterozygotes can be clearly distinguished.

This technique has greatly simplified the study of the inheritance of pseudo-cholinesterase. For instance, it had been suspected previously that a few suxamethonium-sensitive individuals were heterozygotes. We have now seen one such subject with a pseudo-cholinesterase level of 24 units and a dibucaine number of 60. This result is also interesting in that it confirms that heterozygotes can have pseudo-cholinesterase values below 26 units/ml.

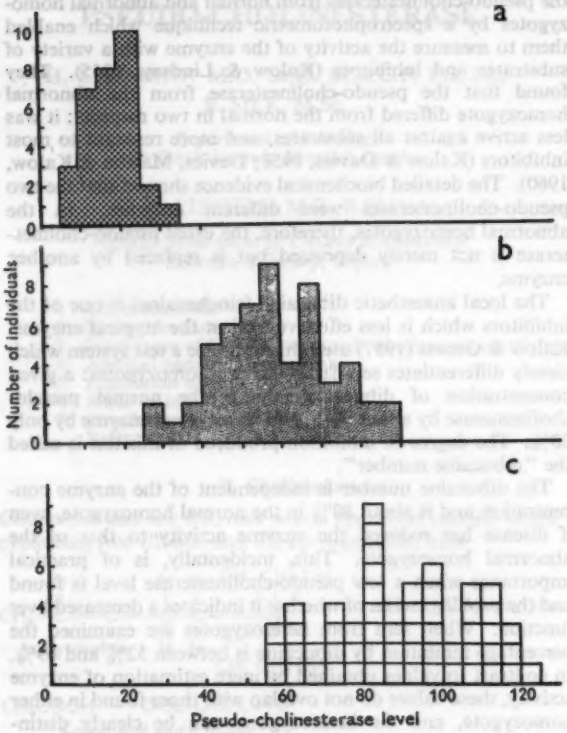
5. Population Surveys

In a population with two allelic genes, a frequency distribution should show three peaks corresponding to the three possible genotypes. Figure 1 demonstrates the pattern found when pseudo-cholinesterase levels are used to identify the genotypes. The following categories are shown:

i. 31 suxamethonium-sensitive propositi chosen because family studies indicated that a low pseudo-cholinesterase level was familial. These should be homozygotes;
ii. 55 parents and children of these propositi. These should all be heterozygotes;
iii. 56 normal adults. Because of the rarity of the abnormal gene, these can be presumed to be normal homozygotes.

Although there is an overlap between the three groups, a trimodal distribution is seen to emerge. However, when the dibucaine technique is used, the three categories are clearly distinct. Figure 2 shows the distribution of dibucaine numbers among 11 propositi and 58 of their relatives.

FIG. 1. Distribution of Plasma Pseudo-Cholinesterase Levels in the Three Genotypes



a: 31 abnormal homozygotes (suxamethonium-sensitive propositi)
b: 55 heterozygotes (parents and children of these propositi)
c: 56 normal adults (presumed to be normal homozygotes because of the rarity of the abnormal gene)

Kalow & Gunn (1959) have estimated the frequency of the three genotypes in a normal Canadian population. They found that between 3% and 4% were heterozygotes, and that abnormal homozygotes occurred with a frequency of about 1 in 2,000. A similar investigation has not been performed in an English population, but the incidence of suxamethonium sensitivity suggests that the genotype frequencies are of the same order.

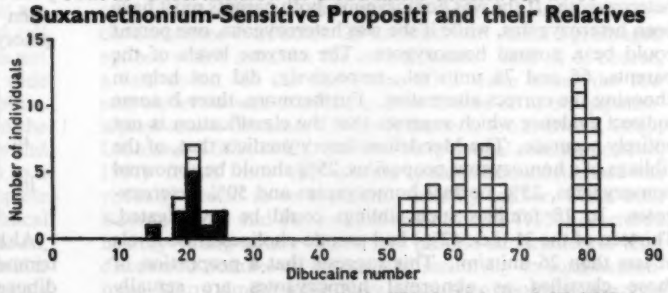
6. Mode of Inheritance

The simplest explanation for the inheritance of pseudo-cholinesterase is that it is determined by two allelic genes (*A*: normal; *B*: abnormal), neither of which is dominant. The two homozygotes will then possess only one of the two enzymes, while both will be present in approximately equal amounts in the heterozygote. This theory is consistent with the inheritance in the majority of families. But there were anomalous findings in two of 18 families, seven of whom were reported by Kalow & Staron (1957), and 11 by Harris, Whittaker, Lehmann & Silk (1960). The two exceptions are shown in fig. 3. In family 1, the simple theory predicts that the mating of a normal and an atypical homozygote should produce only heterozygotes, whereas both offspring appear to be atypical homozygotes. In family 2, the apparent genotypes of II-2 and III-1 show a similar anomaly.

In order to explain these discrepancies, Kalow & Staron (1957) have suggested that there may be two or more allelic genes (A_1, A_2. . .A_n) determining the formation of normal pseudo-cholinesterase at different rates. The extreme of such a series (A_n) would be an allele leading to such a low rate of enzyme formation that it would be virtually inactive. In family 1, the mother had a normal dibucaine number and a total serum pseudo-cholinesterase activity of only 65 units/ml. (normal range: 55–120 units/ml.). These findings would be consistent with the genotype A_1A_n so that the offspring of her marriage with an abnormal homozygote (*BB*) could be heterozygotes with the genotypes A_1B and A_nB. Since A_n would determine the production of an undetectable level of the normal enzyme, the genotype A_nB would appear to be an abnormal homozygote. Similarly, in family 2, the propositus would have the genotype A_nB and could therefore have a child (III-1) with the genotype A_1A_n, an apparently normal homozygote. The only objection is that this mode of inheritance implies the existence of A_nA_n individuals. Such persons, who would have a normal dibucaine number and a genetically determined low serum pseudo-cholinesterase level, have not been reported. Moreover, a number of other genetic hypotheses could be advanced to explain these observed patterns in families. They need not be elaborated here.

Another family has been described by Lehmann, Silk, Harris & Whittaker (1960), which cannot be explained by the theory of Kalow and Staron. The pseudo-cholinesterase level and dibucaine number were normal in one parent and typical for the heterozygote in the other. But five of six offspring had pseudo-cholinesterase levels typical of the abnormal homozygote, while their dibucaine numbers were intermediate between the abnormal homozygote and the heterozygote. Harris and Whittaker, using sodium fluoride as a differential inhibitor, discovered one family in which the enzyme responded differently from all the others examined (Harris, 1961). It is possible that with the aid of further inhibitors yet more pseudo-cholinesterase types may be differentiated. The history of cholinesterase is an example of the use of different inhibitors and substrates in showing that what appeared to be a single enzyme is in fact a group of different catalysts similar in some, but different in other, properties. The subdivision began by dividing "cholinesterase" into "true" and "pseudo-cholinesterase" and, as far as pseudo-cholinesterase is concerned, this process is still going on.

FIG. 2. Distribution of Dibucaine Numbers in 11 Suxamethonium-Sensitive Propositi and their Relatives

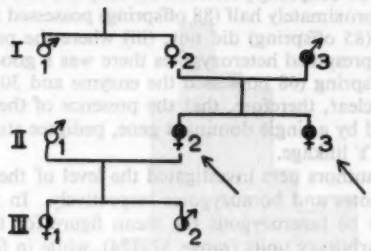


Harris, Whittaker, Lehmann & Silk (1960) by permission of Acta Genetica et Statistica Medica

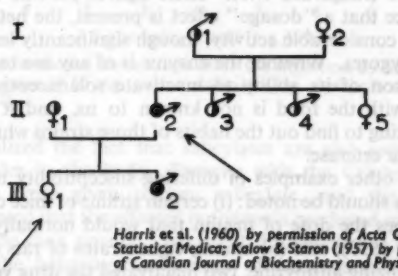
■ suxamethonium-sensitive propositi
□ their relatives

FIG. 3. Two Families Showing an Unusual Mode of Inheritance of Atypical Pseudo-Cholinesterase

Family 1



Family 2



Harris et al. (1960) by permission of Acta Genetica et Statistica Medica; Kalow & Staron (1957) by permission of Canadian Journal of Biochemistry and Physiology

● atypical dibucaine number (presumed abnormal homozygote)
◑ intermediate dibucaine number (presumed heterozygote)
○ usual dibucaine number (presumed normal homozygote)
→ anomalous phenotypes

Family 1 is from Harris et *al.* (1960); family 2 is part of family Y from Kalow & Staron (1957).

7. Conclusion

The importance of pseudo-cholinesterase for the survival of the individual, and even its functions, are unknown. The complete inhibition of the plasma enzyme is certainly compatible with life in normal circumstances, although this may not be true under conditions of stress. For instance, Heymans, Verbeke & Votava (1948) have shown that injections of acetylcholine produce marked cholinergic symptoms in dogs whose pseudo-cholinesterase has been inhibited.

This uncertainty is still more pronounced when one considers not the absence, but the replacement, of pseudo-cholinesterase by an atypical enzyme. Any assessment of the advantages and disadvantages of the various phenotypes can only be speculative. One would certainly expect the weaker hydrolytic power of atypical pseudo-cholinesterase to be a handicap to the abnormal homozygote. This is certainly so if suxamethonium is administered, although the prolonged apnoea produced is rarely fatal. Moreover, this highly artificial situation cannot yet account for any changes in gene frequency.

Since atypical pseudo-cholinesterase is relatively resistant to some inhibitors, but not to all, their effect is more complex. Increased resistance should protect the abnormal homozygote and the heterozygote against differential inhibitors. But the reverse is true of inhibitors which affect both enzymes equally. For example, a concentration of one of these substances which inhibited 50% of the enzyme would reduce the already low pseudo-cholinesterase activity of the atypical homozygote to a potentially dangerous level, and leave the normal homozygote with an adequate esterase activity. Several inhibitors have been found in plant tissues. Heath & Park (1953), for example, demonstrated one such substance in white clover, and suggested that it might be responsible for "bloat", a disease of sheep and cattle. In only one case is it known whether these compounds differentially inhibit the human pseudo-cholinesterase variants. Orgell, Vaidya & Dahm (1958) discovered such an inhibitor in members of the Solanaceae. This was found in potatoes where it was concentrated in the peel; in tobacco leaves; and in the foliage and roots, but not the fruit, of tomatoes. Harris & Whittaker (1959) showed that this substance was a differential inhibitor of the human pseudo-cholinesterases and could even be used, like dibucaine, to identify the phenotypes.

A polymorphism arises from a balance of advantages the allelic genes confer. The low incidence of abnormal homozygotes suggests a disadvantage in natural selection (Dr P. M. Sheppard, personal communication). The normal enzyme is more efficient than the atypical variant in destroying toxic concentrations of acetylcholine and other choline esters. The abnormal enzyme, however, is more resistant to naturally occurring inhibitors. Thus the heterozygote who possesses both enzymes may be at an advantage over both homozygotes in natural selection.

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PHARMACOGENETICS

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A Animal studies

B Human studies
1 Metabolism of salicylates
2 Acatalasia: effects of hydrogen peroxide
3 Porphyria: effects of barbiturates
4 Variations in pupillary responses to Paredrine
5 Primaquine sensitivity
a Clinical features
b Haematological features
c Biochemical studies
d Genetic studies
e Allied conditions
f Geographical distribution of glucose 6-phosphate dehydrogenase deficiency
6 Metabolism of isoniazid
a Therapeutic implications of the polymorphism of isoniazid metabolism in man
7 Some speculations
References

Vogel (1959) introduced the term "pharmacogenetics" and it is defined as the study of genetically determined variations in animal species that are revealed by the effects of drugs.

In the administration of drugs to man we are dealing only with a particular aspect of the general situation which occurs when a foreign chemical is introduced into an organism. Thus, it is well known that, when a deleterious chemical such as an antibacterial agent is added to a culture of bacteria, two types of change occur (Work & Work, 1948). The first is adaptive, appears almost at once, and is characterized by the opening-up of already existing minor metabolic pathways, which are then utilized to a much greater degree than when the normal substrate is available. In other words, bacteria possess enzyme systems which are mostly inactive in the normal environment. The second occurs much more slowly and is due to selection in favour of those mutant strains that possess enzymes capable of neutralizing the foreign compound. Thus, evolutionary forces can give rise to a population with a frequency of resisting enzyme systems very different from those of the ancestral type. A similar situation exists in higher animals, including man, where the study of polymorphic systems provides the best form of approach.

A. ANIMAL STUDIES

The first important study which showed a clear-cut genetic difference in drug action was that of Sawin & Glick (1943), who investigated the atropine esterase activity of the serum of rabbits by assessing its ability to destroy atropine sulphate in vitro. It was found that only certain strains of rabbit possessed atropine esterase and that their ability to form it was genetically controlled. The evidence for this was as follows: (i) in matings of parents lacking atropine esterase none of the 69 offspring showed any evidence of the presence of this enzyme; (ii) where one parent possessed the enzyme, but the other lacked it, two types of family were found—one type in which all 37 offspring possessed the enzyme, and the other in which approximately half (88 offspring) possessed the enzyme and half (85 offspring) did not; (iii) where the parents were both the presumed heterozygotes there was a good 3:1 ratio in the offspring (68 possessed the enzyme and 30 lacked it). It seems clear, therefore, that the presence of the enzyme is controlled by a single dominant gene, pedigree studies ruling out X or Y linkage.

These authors next investigated the level of the enzyme in heterozygotes and homozygotes respectively. In 25 animals known to be heterozygous the mean figure for the enzyme was 107 arbitrary units (range 52–174), while in four known to be homozygous for the presence of the enzyme the mean was 271 (range 232–348). These figures provide convincing evidence that a "dosage" effect is present, the heterozygotes having considerable activity, though significantly less than the homozygotes. Whether the enzyme is of any use to the rabbit by reason of its ability to inactivate solanaceous alkaloids eaten with the food is not known to us, and it would be interesting to find out the habits of those strains which possess atropine esterase.

Two other examples of differing susceptibility to drugs in animals should be noted: (i) certain strains of mice can survive 300 times the dose of insulin that would normally be lethal (Chase, 1950), and (ii) among eight strains of rats capable of metabolizing antipyrine, two inactivated the drug very slowly, whereas the other six dealt with it rapidly (Brodie, 1952).

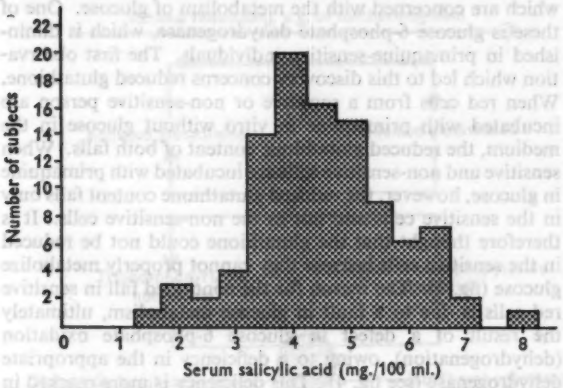
B. HUMAN STUDIES

1. Metabolism of Salicylates

In the metabolism of sodium salicylate there are certain indications in the literature (Coburn, 1943; Smith, Gleason, Stoll & Ogorzalek, 1946; Carr, Mouratoff & Batterman, 1958) that considerable variations exist in the serum salicylic acid concentration after the administration of sodium salicylate by mouth to apparently normal people. Further support for this view was given by investigating the fate of ^{14}C-labelled salicylic acid in four human subjects, which resulted in the finding of a great variation (10–85% of the ingested dose) in the recovery both of salicylic acid and salicyluric acid in the urine (Alpen, Mandel, Rodwell & Smith, 1951). On the other hand, Evans (unpublished, 1960) has clearly shown that the administration of a standardized dosage of sodium salicylate to 100 tuberculous subjects (fasting, and without any therapy for the previous 48 hours) gives a normal distribution for the serum salicylic acid three hours after drug ingestion (fig. 1). Therefore, despite the variability suggested in the literature quoted, it seems highly improbable that, in man, there is any important polymorphism in the way the drug is handled.

Salicylates can, however, be used for the investigation of certain spontaneous disease states known to be genetically determined. For example, there are several types of familial non-haemolytic jaundice. One of these, known as the Crigler-Najjar syndrome (Schmid, 1960) (inherited as an autosomal recessive trait) is due to the absence of glucuronyl transferase, the glucuronyl group being obtained from uridyl diphosphate. This enzyme, present in the liver, is responsible for converting bilirubin into its glucuronide, and Childs, Sidbury & Migeon

FIG. 1. Distribution of Concentrations of Serum Salicylic Acid in 100 Tuberculous Subjects



Distribution is shown three hours after the ingestion by patients in the fasting state of 50 mg. sodium salicylate/kg. metabolically active mass ($wt.^{0.7}$).

(1959) utilized the fact that salicylates are also secreted as glucuronides in the body. Patients with the Crigler-Najjar syndrome were given sodium salicylate, and the results demonstrated that the affected individuals produced much less glucuronide than normal. No great difference was detected between the heterozygous parents of patients and normal subjects. Thus, a drug can be used as a test object to exhibit the metabolic defect present in certain types of disease. The same workers also used trichloroethanol and cortisol in a similar way because these too are conjugated with glucuronide in normal people, but not in patients with Crigler-Najjar jaundice.

2. Acatalasia: Effects of Hydrogen Peroxide

The condition was discovered by the otorhinolaryngologist Takahara (1952), and all the cases so far reported have been in Japanese. The way the condition came to light is a model of clinical observation. Takahara was cleaning out the maxillary sinus of a patient, with hydrogen peroxide, when he noticed that no frothing occurred on the raw surface and that the free blood adjacent to it turned black; these effects proved reproducible even with new bottles of hydrogen peroxide. Takahara speculated that there might well be in the blood a deficiency of the enzyme catalase that normally degrades hydrogen peroxide, thereby preventing it from degrading haem proteins. The catalase activity of the blood of his patient was, therefore, estimated and found to be nil. It is thought that, as haemolytic streptococci and certain pneumococci themselves produce hydrogen peroxide, in a patient lacking catalase the haemoglobin of the blood reaching a lesion is oxidized, so that the infected area is deprived of its normal oxygen, and necrosis occurs. In this situation the bacteria multiply, the hydrogen peroxide production increases and a vicious circle is established.

Since 1952 there have been described 38 affected individuals occurring in 17 Japanese families. The sex distribution is normal. Consanguinity of parents was present in no less than 16 of the families and in no instance is there a record of acatalasic parents giving rise to affected offspring. This strongly supports the view that the condition is inherited as an autosomal recessive trait. Known heterozygotes have blood catalase activity intermediate between that of normal subjects and the zero value of those affected (Nishimura, Hamilton, Kobara, Takahara, Ogura & Doi, 1959) (see fig. 2). An analagous disorder with a similar type of inheritance occurs in certain breeds of dogs and guinea-pigs (Allison, ap Rees & Burn, 1957; Radev, 1960).

Reviewing the subjects with acatalasia in the light of a knowledge of their defect, two points of interest are worth noting: (i) while the disability is usually exhibited before the age of ten years, yet about half those who lack the enzyme have no symptoms whatsoever; (ii) when the disorder is manifest there is a predisposition to dental sepsis and ulceration of the gums, leading eventually to the loss of all the teeth. Granulating tumours and destructive lesions sometimes develop in the paranasal sinuses and nasopharynx. However, when the patient becomes edentulous the condition regresses and no further trouble is experienced.

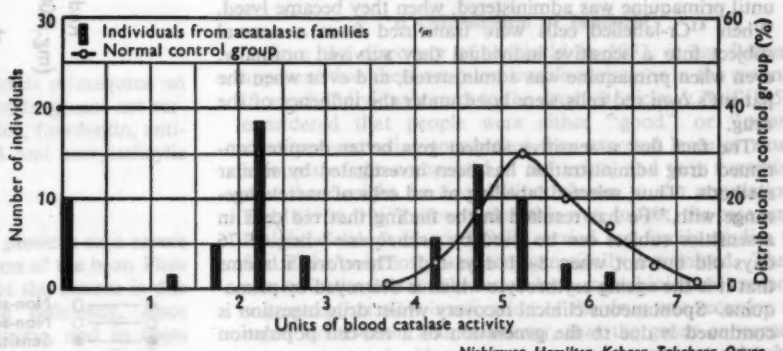
It is interesting to speculate whether the defect in acatalasia lies in the failure to produce the haem-like prosthetic group which the enzyme is known to possess. This failure cannot be general as the patients are not anaemic and, in fact, the lesions are confined to the mouth, and ill-health is not of a severe degree. It has been suggested that many of the functions of catalase can be carried out by peroxidases, and acatalasic subjects are known not to have a general deficiency of these enzymes—though there might be a local lack of them in the mouth. Clearly, this matter needs further investigation.

We are indebted to the review of Wyngaarden & Howell (1960) for much of the information in this section.

3. Porphyria: Effects of Barbiturates

Attacks of porphyria are not infrequently precipitated by taking barbiturates, and this occurs chiefly in the South African form of the disease.

FIG. 2. Distribution of Catalase Values for Members of Acatalasic Families, and a Percentage Distribution Curve of Values from a Normal Control Group


Individuals from acatalasic families
Normal control group
Number of individuals
Distribution in control group (%)
Units of blood catalase activity

Nishimura, Hamilton, Kobara, Takahara, Ogura & Doi (1959) by permission of Science

4. Variations in Pupillary Responses to Paredrine

Blue-eyed people are said to be more sensitive than brown-eyed to the action of Paredrine (Wells, 1958), and mongols are particularly sensitive to atropine (McKusick, 1957). However, as far as we are aware, nothing further is known about the reasons for these phenomena.

5. Primaquine Sensitivity

Haemolytic anaemia was recognized as an occasional complication from the use of pamaquin when this was introduced into therapeutics in 1926 as an agent against the ex-erythrocytic forms (i.e., those in the sexual phase) of *Plasmodium vivax*. At first this was thought to be due to a hypersensitive or immune mechanism, but an antibody was not discoverable in the blood, and no explanation could be found for the occurrence of haemolytic anaemia in these sensitive subjects. With the advent of wide-spread treatment of malaria with the very similar drug primaquine during the Second World War, more cases of haemolytic anaemia were studied.

a. Clinical Features

When a sensitive subject is given 30 mg. of primaquine daily, he does not develop haemolytic anaemia for two or three days. Thereafter his urine gradually turns dark, muscular pains occur and anaemia and possibly jaundice appear. Discontinuing the drug results in a return to normal after a few weeks. However, if the symptoms are not severe and primaquine ingestion is continued, the patient will, surprisingly, also gradually improve. It will be seen later that this is a most important observation.

b. Haematological Features

On investigation it is obvious that the anaemia is haemolytic, the dark urine being due to haemoglobin and increased urinary urobilinogen. There is no evidence of deficient blood production and there is no haemorrhage. The Coombs' test is negative, the erythrocyte fragility is normal, and there is no spherocytosis. The striking finding in the blood is the presence of Heinz bodies (denatured protein) in the red cells, and after the condition has been present for some days a reticulocytosis occurs (Beutler, 1959, 1960).

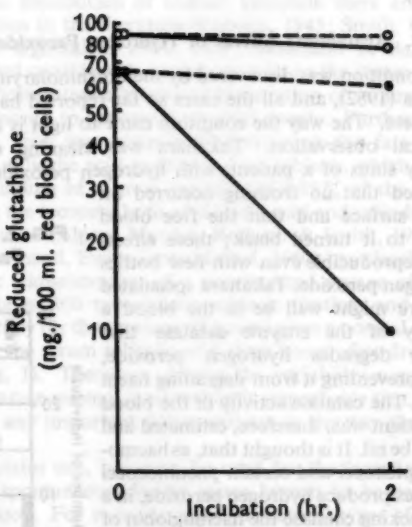
Dern, Weinstein, LeRoy, Talmage & Alving (1954) transfused ^{51}Cr-labelled red cells from sensitive subjects into non-sensitive recipients. The survival of these cells was normal until primaquine was administered, when they became lysed. When ^{51}Cr-labelled cells were transfused from a normal subject into a sensitive individual they survived normally, even when primaquine was administered, and even when the patient's own red cells were lysed under the influence of the drug.

The fact that a sensitive subject gets better despite continued drug administration has been investigated by similar methods. Thus, selected labelling of red cells of narrow age-range with ^{59}Fe has resulted in the finding that red cells in a sensitive subject can be lysed by primaquine when 63–76 days old but not when 8–21 days old. Therefore, it seems that it is the ageing erythrocyte which is destroyed by primaquine. Spontaneous clinical recovery whilst drug ingestion is continued is due to the generation of a red-cell population with a low mean age.

c. Biochemical Studies

The normal red cell is known to possess enzyme systems which are concerned with the metabolism of glucose. One of these is glucose 6-phosphate dehydrogenase, which is diminished in primaquine-sensitive individuals. The first observation which led to this discovery concerns reduced glutathione. When red cells from a sensitive or non-sensitive person are incubated with primaquine in vitro without glucose in the medium, the reduced glutathione content of both falls. When sensitive and non-sensitive cells are incubated with primaquine in glucose, however, the reduced glutathione content falls only in the sensitive cells and not in the non-sensitive cells. It is therefore thought that the glutathione could not be reduced in the sensitive cells because they cannot properly metabolize glucose (fig. 3). The reason for the continued fall in sensitive red cells is due to a fault in glucose metabolism, ultimately the result of a defect in glucose 6-phosphate oxidation (dehydrogenation), owing to a deficiency in the appropriate dehydrogenase (see fig. 4). This deficiency is more marked in the older cells. Further investigation of normal and sensitive subjects has shown that there is probably a quantitative lack rather than a qualitative change in the enzyme (Childs & Zinkham, 1959; Kirkman, 1959; Marks, Szeinberg & Banks, 1961). It has not been definitely proved that the mean red-cell concentration of glucose 6-phosphate dehydrogenase is higher in a sensitive subject after he has recovered clinically while still receiving primaquine.

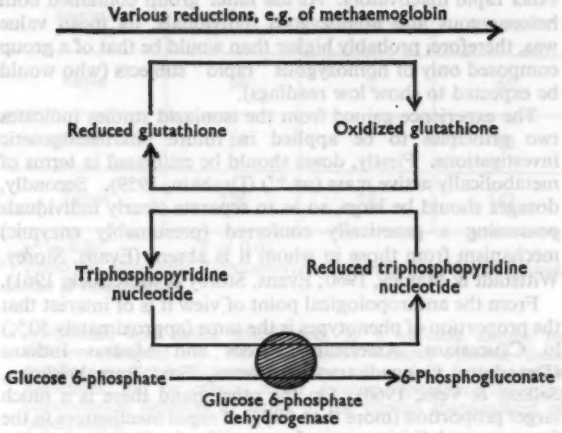
FIG. 3. Effect on Reduced Glutathione of Incubating Sensitive and Non-Sensitive Red Cells with Primaquine Diphosphate (10 mg./ml.) in the Presence of Glucose



Beutler (1960) by permission of McGraw-Hill Book Co. Inc.

○——○ Non-sensitive red cells incubated without primaquine
○——○ Non-sensitive red cells incubated with primaquine
●——● Sensitive red cells incubated without primaquine
●——● Sensitive red cells incubated with primaquine

FIG. 4. Site of the Metabolic Defect in Primaquine Sensitivity



Site of defect is indicated by shaded area.

d. Genetic Studies

Males are readily assignable to either the sensitive or non-sensitive group as a result of both glutathione and glucose 6-phosphate dehydrogenase studies. Women do not give quite so clear-cut a division, and intermediate values are observed with either method of assessment. Childs, Zinkham, Browne, Kimbro & Torbert (1958) studied 16 families including 30 sibships. Male-to-male transmission was rare, appearing only once. The usual picture was that of an "intermediate" female who produced sensitive sons or "intermediate" daughters.

Pedigree analysis and the sex differences led to the conclusion that the gene controlling the presence or absence of glucose 6-phosphate dehydrogenase is on the X chromosome, i.e., the trait is a sex-linked "dominant", the penetrance of the gene being incomplete because "intermediate" daughters and sensitive sons are sometimes found to have apparently normal parents. The gene frequency figure of 0.14 gives a frequency of heterozygotes of 0.24 and homozygotes of 0.02. This agrees well with the data obtained from a survey of 144 males and 184 females of negro race, attending medical and paediatric out-patient clinics, of whom 15% of males and 2% of females were in the sensitive group.

e. Allied Conditions

i. Other Drugs

Other drugs which have the same effect as primaquine on cells deficient in glucose 6-phosphate dehydrogenase are certain sulphonamides, phenacetin, acetanilide, furadantin, antipyrin, probenecid, *p*-aminosalicylic acid and acetylsalicylic acid.

ii. Favism

This disorder, known from antiquity, presents as a severe haemolytic anaemia following the ingestion of the bean *Vicia fava*. There is considerable evidence that the disease is due to glucose 6-phosphate dehydrogenase deficiency, since sufferers show lower assays of this enzyme, and in them glutathione (GSH) stability is lower than normal. Furthermore, if they take primaquine, haemolytic anaemia develops, though this is not as severe as with ingestion of the bean, suggesting that some other factor is present in favism. A familial incidence has long been recognized and appropriate studies have shown that the method of inheritance of favism is the same as that of primaquine sensitivity.

iii. Moth-balls

A similar haemolytic anaemia associated with abnormal GSH stability levels has been observed in some subjects following the ingestion of moth-balls (Zinkham & Childs, 1958). This may occur in children, and in adults as a perversion of appetite (e.g., during pregnancy).

iv. Neonatal Jaundice

Doxiadis, Fessas, Valaes & Mastrokalos (1961) have recently pointed out that in Greece there is a high proportion of cases with severe neonatal jaundice which cannot be explained by either Rh or ABO incompatability; moreover, the jaundice has been shown not to be due to haemoglobinopathy or inadequate conjugation of bilirubin. The authors have demonstrated that there is a high incidence of glucose 6-phosphate dehydrogenase deficiency among cases of neonatal jaundice of unknown causation, as compared with the incidence in a hundred cord bloods taken at random. The precipitating cause of the jaundice is not known, but it is speculated that drugs given to the mother might be a factor. The condition is treatable by exchange transfusion.

f. Geographical Distribution of Glucose 6-Phosphate Dehydrogenase Deficiency

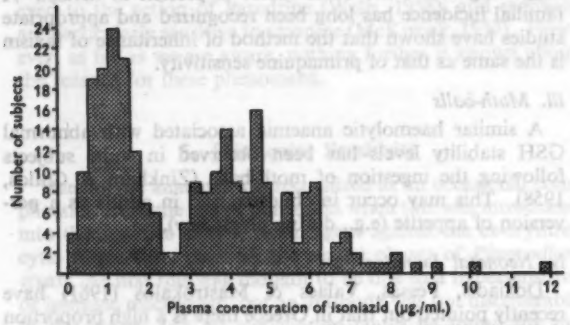
A high incidence of the basic defect (10% or more) has been reported amongst American negroes, Sardinians, Iraqis, Persians, Indians, Indonesians, Thais and Formosans. Lower incidences occur in populations of adjacent areas. The reasons for the existence of the polymorphism are unknown. The world distribution suggests that glucose 6-phosphate dehydrogenase deficiency, like that of haemoglobin S, may be in some way less favourable to the proliferation of malaria plasmodia. In support of this Allison & Clyde (1961) found that parasite rates and densities of *Plasmodium falciparum* were significantly lower in young African children with erythrocyte glucose 6-phosphate dehydrogenase deficiency than in children with normal enzymes. The degree of protection seems likely to be of the same order as that conferred by the sickle-cell trait.

Most of the information in this section has been obtained from Beutler (1959, 1960).

6. Metabolism of Isoniazid

Since the discovery of isoniazid in 1952, it has become apparent that there are considerable variations in the manner in which individuals react to the drug. Bönicke & Reif (1953) considered that people were either "good" or "poor" excretors according to whether they excreted large or small amounts of the compound in the urine, and they suggested that there might be two distinct classes in the population. Biehl (1956, 1957) showed that this was, in fact, the case, and Knight, Selin & Harris (1959) carried out family studies to find if the polymorphism were genetically determined. Their results in the 20 families tested by a microbiological method suggested that slow inactivation of the drug was recessive to rapid inactivation and, moreover, that the genes concerned were autosomal. However, there was a very considerable

FIG. 5. Distribution of Plasma Isoniazid Concentrations in 267 Members of 53 Families



Evans, Manley & McKusick (1960) by permission of British Medical Journal

The distribution is shown at six hours after oral administration of 9.7 mg. isoniazid/kg. body-wt.

overlap in the two classes so that the scoring of some individuals was doubtful. Evans, Manley & McKusick (1960) therefore carried out a much bigger investigation and used a more accurate, chemical method of assay. Taking 484 people, they estimated the plasma concentration of isoniazid six hours after a single oral dose of 10 mg./kg. body-weight, and found a bimodal distribution with an antimode at 2.5 μg./ml. There was no significant difference in the proportions of the two phenotypes between the two Caucasian and the two negro populations of which this series consisted.

Of the 484 individuals studied, 267 were members of 53 complete, two-generation, Caucasian family units, and fig. 5 is a histogram showing the isoniazid distribution for these subjects. It will be seen that approximately half were slow inactivators, and half rapid inactivators, and analysis of the mating types confirmed that the slow inactivator character was indeed recessive (Table I).

Of great additional interest was the fact that a dosage effect was demonstrable. Thus, in the individuals showing the dominant character the mean plasma isoniazid concentration of known heterozygotes was different from that of all other rapid inactivators. As the latter group contained both heterozygous and homozygous individuals, its mean value was, therefore, probably higher than would be that of a group composed only of homozygous "rapid" subjects (who would be expected to show low readings).

TABLE I. Observed Numbers of Children of each Phenotype, Compared with those Expected on the Hypothesis that Slow Inactivation of Isoniazid is an Autosomal Recessive Character

Phenotypic matings	Number of matings	Number of children	Number of children of each phenotype				χ^2	D.F.‡
			R*		S†			
			Expected	Observed	Expected	Observed		
S × S	17	54	nil	4	54	50	—	—
R × S	23	67	38.88	40	28.10	27	0.075	1
R × R	13	38	31.30	31	6.68	7	0.018	1
	53	159		75		84	0.093	2

$P > 0.95$

*R: rapid inactivators
†S: slow inactivators
‡D.F.: degrees of freedom

The experience gained from the isoniazid studies indicates two principles to be applied in future pharmacogenetic investigations. Firstly, doses should be estimated in terms of metabolically active mass ($wt.^{0.7}$) (Drabkin, 1959). Secondly, dosages should be large, so as to separate clearly individuals possessing a genetically conferred (presumably enzymic) mechanism from those in whom it is absent (Evans, Storey, Wittstadt & Manley, 1960; Evans, Storey & McKusick, 1961).

From the anthropological point of view it is of interest that the proportion of phenotypes is the same (approximately 50%) in Caucasians, American negroes and Madras Indians (Devadatta, Gangadharam, Andrews, Fox, Ramakrishnan, Selkon & Velu, 1960). On the other hand there is a much larger proportion (more than 90%) of rapid inactivators in the Japanese and Eskimo populations (Harris, Knight & Selin, 1958; Mitchell, Riemensnider, Harsch & Bell, 1958; Armstrong & Peart, 1960). The advantage of the dominant character is unknown, but it would appear that its possession is particularly advantageous in the Far Eastern and Arctic environments.

Sunahara *et al.* (1960) have shown, using a very refined microbiological technique, that there is a trimodal distribution of serum isoniazid in Japanese tuberculous patients. This finding suggests that their technique would be able to separate out the three genotypes in the population, and family studies by Sunahara (1961) show that this is in fact so.

a. Therapeutic Implications of the Polymorphism of Isoniazid Metabolism in Man

There are three aspects of the treatment of tuberculosis with isoniazid in which the inactivator phenotype of the patient might be of importance. These are: (i) the development of polyneuritis with long-term exhibition of the drug; (ii) the response of tuberculous disease to isoniazid treatment; (iii) the development of isoniazid-resistant tubercle bacilli.

The recent study of Devadatta *et al.* (1960) gives a clear answer to the first consideration. They say that, of 19 patients who developed peripheral polyneuritis on a dose of 8–9 mg./kg./day, only two were rapid inactivators, while 17 were slow inactivators of isoniazid; this occurred in a population where the incidence of the two phenotypes is close to 50% (see Table II).

With regard to the response of the tuberculous disease to treatment with the drug, the evidence is very unsatisfactory. Mitchell *et al.* (1958) suggest that the trend is for patients with higher serum isoniazid concentration to have a quicker reversal of infectiousness than those with lower serum concentrations; further, that the frequency of reversal also tends to be higher in the slow inactivator (i.e., high serum isoniazid concentration) group. However, all these results are suspect because *p*-aminosalicylic acid was administered simultaneously with isoniazid, both in treatment and in the procedure to determine the isoniazid inactivator phenotype. McDermott (1960) states that:

> In 1954, Schmidt and his associates . . . published elegant chemical studies of the pharmacology of isoniazid and showed that it is

TABLE II. The Development of Polyneuritis in Patients on Isoniazid Therapy

Inactivator phenotype	No polyneuritis	Polyneuritis	Totals
Rapid	58	2	60
Slow	66	17	83
Totals	124	19	143

$\chi^2 = 8.87$ $P < 0.01$

Based on *Devadatta, Gangadharam, Andrews, Fox, Ramakrishnan, Selkon & Velu* (1960)

acetylated, and hence inactivated, to a variable degree by different subjects . . . In the present writer's opinion, these excellent studies by Schmidt have been given (not by him or his associates, but by others) a far greater importance than the phenomenon deserves in terms of the clinical management of tuberculosis.

With regard to the development of isoniazid-resistant strains of tubercle bacilli, the information given by Biehl (1957) conclusively shows that there is no association between the isoniazid inactivator phenotype and the development of tubercle bacilli resistant to the drug. It is quite clear that further and extensive investigations must be made to see what is the influence of the inactivator phenotype on the outcome of tuberculous disease treated with isoniazid. This is all the more important in view of the fact that great stress is laid on chemotherapy, particularly on isoniazid (sometimes given alone), in under-developed countries (Dormer, Harrison, Swart & Vidor, 1959).

7. Some Speculations

It is of course quite possible that drugs other than those discussed in this symposium, viz. salicylates, hydrogen peroxide, isoniazid, atropine and succinylcholine (see Lehmann, Silk & Liddell, 1961), may exhibit genetically determined differences in their metabolism or action within human populations. There is a clue that such a difference is, in fact, present for the anti-thyroid drug thiouracil, and derivatives, because these, like phenylthiocarbamide, exhibit a taste threshold difference in human beings. Further, we know from the studies of Kitchin, Howel Evans, Clarke, McConnell & Sheppard (1959) that the phenomenon of varying taste sensitivity to phenylthiourea has a certain amount of relevance to the incidence of spontaneous thyroid disease in human populations. (See Kitchin & Howel Evans, 1960.)

Motulsky (1957) points out that there may be a difference in the reaction to drugs between racial groups, and when other environmental variables are equal these will usually have a genetic basis. He also says that "Genetically conditioned drug reactions not only are of practical significance but may be considered pertinent models for demonstrating the interaction of heredity and environment in the pathogenesis of disease."

Cooper & Friedman (1958), on the basis of a purely biochemical investigation of the enzyme which oxidizes chloral hydrate to trichloroacetic acid in the body, point out that this investigation emphasizes ". . . a hitherto lightly regarded facet of drug metabolism studies, namely, the use of a drug as a substrate to uncover new enzyme systems." This leads us back to our opening remarks, because we can now consider the relevance of bacterial studies to those of drug-determined polymorphisms in man. Thus Williams (1959) states that it seems unlikely that an organism will possess a special mechanism to deal with a synthetic organic compound which has not yet been synthesized. This author, at the end of his large and informative volume, concludes by quoting Brodie (1956) who says:

. . . we can consider the possibility that the enzyme systems . . . of mammals which have a major role in limiting the duration of action of drugs have developed by a process of evolution to protect the organism from a multitude of foreign compounds ingested in food as well as produced by micro-organisms in the intestines.

We can go on to say that these properties have developed into a polymorphic system in man for many drugs, and that the proportions of the phenotypes differ in different races. For instance, about 10% of negroes possess a glucose 6-phosphate dehydrogenase deficiency, while as regards isoniazid there is a very great preponderance of rapid inactivators in the Far Eastern populations. Apart from their strictly clinical and practical application, these considerations are of great importance to biochemical genetics because a drug has usually a quite well-known chemical structure, and as a rule methods are readily available for determining its concentration both in vitro and in body liquids.

Drugs are useful tools, therefore, with which to investigate fundamentals in biochemical genetics, particularly enzyme systems which are controlled by allelic genes. The study of Kalow and his collaborators (Davies, Marton & Kalow, 1960) on cholinesterases, and the studies of Kirkman (1959) on glucose 6-phosphate dehydrogenase, are typical examples of the way in which a discovery of variations in the clinical responses to drugs can lead eventually to important observations in the field of enzyme chemistry.

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MULTIFACTORIAL INHERITANCE IN RELATION TO NORMAL AND ABNORMAL HUMAN TRAITS

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1 Measures of resemblance between relatives
2 An example of polygenic inheritance in man in which theoretical values are closely realized
3 Stature
4 General intelligence and mental deficiency
5 Arterial pressures and essential hypertension
6 The genetic component in the causation of common diseases
References

The concept of multifactorial inheritance was clearly formulated by Mendel himself. He had crossed two varieties of flowering beans, one having purple flowers and the other white. The F_1 generation was intermediate, and in F_2 there was a range of colours from purple to white. He suggested that this might be owing to the additive action of more than one gene pair. This kind of gene action was first demonstrated by Nilsson-Ehle (1909).

In more recent times the term "polygenic" has been widely used, though in two ways. Some writers, for example Stern (1960), equate polygenic and multifactorial inheritance, and treat as polygenic inheritance any instance in which the observed character is determined by the combined action of more than one gene pair. These may be as few as two. Others, for example Darlington & Mather (1949), describe inheritance as polymeric if, as just stated, the action of the genes is similar and supplementary, but require a third criterion for polygenic inheritance. This is that the effect of any single gene shall be small in relation to the total variation. Thus all polygenic inheritance is polymeric, but not all polymeric inheritance is polygenic. Inheritance which is polymeric without being polygenic leads to discontinuity in frequency distribution, whereas polygenic inheritance yields continuously distributed frequency curves. The distinction is primarily a practical one. If only two, or a very few, gene pairs are concerned, or if the contribution of one gene is large in proportion to the total variation, the classical methods of Mendelian analysis can be used, at least in experimental forms. With polygenic inheritance in the more restricted sense, quite different methods of biometrical analysis are needed.

One point must be made clear. It is by no means supposed that the relatively small effects of the genes making up polygenic systems need be their only effects. It has been known for 50 years that ordinary major genes, whose principal action is to produce marked qualitative differences, very often have ancillary effects on many metrical characters. Many of the major abnormal genes known in man have small average effects on such continuously distributed traits as stature or intelligence. Thus, the side-effects of major genes play their part in determining polygenic variation. It is impossible to say how much. Some might hold that the contribution is considerable; others, that it is likely to be small compared with the sum total of the contribution of the genes whose only action is minor.

The basis for the genetic treatment of continuous variation was provided by Fisher (1918) in a classical paper. He also showed that, so far from the results of the early biometricians contradicting Mendelian theory, they were in full harmony with it; and, moreover, those results could not be rationally explained in any other way. Since then there has been much development in the methods of quantitative genetics. They are complex, however, and their application to man is fraught with difficulty. With man we cannot experiment, merely observe. Even in conventional Mendelian analysis, that is, in the study of single genes, this leads to much complication. Elaborate statistical methods have to be employed for tasks which in the laboratory are simplicity itself. But, as just mentioned, even in the experimental field the methods of biometrical genetics are very complex, so that in man we are restricted to a fraction of what can be done with the animals and plants of the laboratory or the farm. We are in fact limited to little more than measures of resemblance between relatives, and, furthermore, for obvious practical reasons, usually to the resemblances between close relatives. Knowledge even on this restricted scale is, however, very useful, both in the study of normal variation and in assessing the role of heredity in the causation of common diseases.

1. Measures of Resemblance Between Relatives

As Fisher (1918) originally showed, the theoretical measures of resemblance between relatives, given polygenic inheritance, and on certain simplified assumptions, turn out to be very simple. The regressions of child on parent, or parent on child, or sib on sib, are equal to the number of genes in common, and this is also true for more remote relationships. The expression, genes in common, means identical genes derived from the same ancestor genes. Genes not derived from the same ancestor genes may be the same or different, depending on their frequencies in the population. The proportion of genes in common, and hence the regressions, for a number of fairly close relationships are shown in Table I.

As the two regressions are usually equal, these are also the correlation coefficients. The conditions under which the theoretical values are realized are: (i) that inheritance alone

TABLE I. Genes in Common

Relationship to a given subject	Proportion of genes in common
Parent, child, sib	$\frac{1}{2}$
Identical twin	1
Fraternal twin	$\frac{1}{2}$
Grandparent, grandchild, uncle, aunt, nephew, niece, half-sib	$\frac{1}{4}$
First cousin	$\frac{1}{8}$
Second cousin	$\frac{1}{32}$

is involved; (ii) that the gene pairs do not display dominance and recessiveness, i.e., that the heterozygote is intermediate between the two homozygotes; (iii) that values for husband and wife are uncorrelated, namely, that there is no assortative mating. It will be noted that the correlations are the same as those for a single gene pair without dominance.

Assortative mating increases the measures of resemblance, but can be allowed for. Dominance raises special problems. On the simplified model the frequencies of the genes are immaterial, but with dominance the correlations depend upon gene frequency. Thus, if a is the frequency of the recessive allele, the parent-child correlation becomes

$$\frac{a}{1+a}$$

and the sib-sib correlation

$$\frac{1+3a}{4(1+a)}$$

The general effect of dominance is to reduce the resemblance between relatives, but to do so less for sibs than for other relatives. In fact, for any gene frequency the fall from the value of ½ is half as much for sibs as it is for parents or children.

2. An Example of Polygenic Inheritance in Man in which Theoretical Values are Closely Realized

A series of elegant studies by Holt provide much the best human example of multifactorial inheritance, with hardly any environmental component and with a minimum of complication of any other kind.[1] It would in fact be hard to equal, even in experimental material. A convenient summary and bibliography has recently appeared (Holt, 1961). The finger-print ridges are counted according to specified rules, the counts for the ten fingers being added together to give a total count, which is the variable considered here. One great advantage of this character is that the number of ridges is finally determined by the fourth month of foetal life, so there are no troublesome allowances to make for differences in age. There is a sex difference of moderate size, but allowance for this makes practically no difference to the calculations. Table II shows the observed values of the correlations, compared with the theoretical values given above.

It will be seen that the theoretical values are closely realized, the only difference which is significant being that for identical twins. It can thus be deduced that inheritance is almost wholly responsible for differences in total ridge-count. There is no evidence of assortative mating, so this complication is absent. Turning to dominance effects, these can be examined by an ingenious method due to Penrose (1949). Use is made of the regression of child on mid-parent, that is, the mean measurement of the two parents. The theoretical regression of child on mid-parent on the simplified model is 1, for all the child's genes come from father and mother, so that the mid-parent value is the best estimate. The regression of mid-parent on child is, however, ½, because only half the genes of the two parents are identical with those of the child, while the other half are, on the average, those of the general population. The correlation coefficient is therefore $\frac{1}{\sqrt{2}}$, or 0.71. In Holt's material (Holt, 1961) the observed correlation with 301 pairs was 0.67 ± 0.03, agreement being excellent. Penrose points out that dominance and recessiveness of the genes would not only lower the regression of child on mid-parent from the theoretical 1, but would also make the regression non-linear. In Holt's data, the difference from 1 is not significant and there is no significant departure from linearity. Hence it can be deduced that, in their relation to each other, the gene pairs determining total ridge-count do not display any appreciable degree of dominance and recessiveness.

It should be mentioned that the frequency distribution of ridge-counts departs from the normal Gaussian form. There is evidence of concentrations of observations at a few particular points. This is thought to indicate that a few genes are producing an effect which is considerable in comparison with the total variation. So far, however, it has not proved possible to devise a model which fits satisfactorily but, in spite of this feature, ridge-counts remain a most satisfying example of multifactorial inheritance in man. It is very valuable to have human data which fit the simplest theoretical model so perfectly. Doubtless, in time, the observations will be extended to rather more distant relatives. It will also be valuable in the future to include finger-print ridge-counts in measurements made on relatives; they would provide a kind of sheet-anchor when the inevitable complexities that arise with other measurements are being analysed.

[1] See Holt, p. 247 of this number of the Bulletin.—Ed.

TABLE II. Finger-Print Ridge-Counts: Resemblances Between Relatives

Relationship	Number of pairs	Observed correlation	Theoretical correlation
Parent-child	602	0.50 ± 0.03	0.5
Sib-sib	642	0.49 ± 0.04	0.5
Identical twins	80	0.95 ± 0.01	1.0
Fraternal twins	92	0.49 ± 0.08	0.5
Husband-wife	149	0.05 ± 0.( )	0.0

Data in this table are from Holt (1961) and other papers by the same author. See Holt, p. 247 of this number of the Bulletin.—Ed.

3. Stature

In the example discussed above, environmental influences were playing practically no part, but this is probably very unusual with human measurements. With most multifactorial variation, environment is influencing the result to an appreciable extent, and it may be to a large extent. This introduces complications. It has been shown, however, that, given suitable bodies of data, analyses can be made (Fisher, 1918; Mather, 1949). The difference produced by a gene pair is defined as $2d$, and the sum of squares of all such differences as $D = S(d^2)$. H is the dominance component, which is $S(h^2)$, where h_a is the departure of manifestation in the heterozygote from the midpoint of AA and aa. The non-heritable component of variation is denoted by E. Then the parent-offspring correlation is

$$\frac{\frac{1}{2}D}{\frac{1}{2}D + \frac{1}{4}H + E}$$

and the sib-sib correlation is

$$\frac{\frac{1}{2}D + \frac{1}{4}H}{\frac{1}{2}D + \frac{1}{4}H + E}.$$

When dominance effects and environmental effects are absent, these expressions reduce to $\frac{1}{2}$, as in the preceding section.

If data are available for estimating the two correlations, the relative magnitudes (which are all that is required) of D, H and E can be estimated. Fisher and Mather use data provided by Pearson & Lee (1903) on stature. The two correlations are respectively 0.4180 and 0.4619, which gives $H = 0.4201\ D$, and $E = -0.0069\ D$. The non-heritable variation is thus very small, the estimate, in fact, becoming negative owing to sampling error. Taking E as zero, the variation due to dominance is 17%, 83% being due to heritable factors (though a part of the dominance effects is included in this figure). There is, however, a correlation between the statures of husbands and wives. Allowing for this, Fisher estimates that the contributions of H and D are approximately in the ratio 1:3.

It is remarkable how often those seeking an example to illustrate the genetics of human metrical characters go back fifty years to the observations of Pearson and Lee. There is, in fact, a great shortage of such data. The samples must be very large indeed if reasonably accurate estimates are to be made. The sampling errors of the estimates quoted above are very large. It is also difficult to secure unbiased samples. Nevertheless, the task is not impossible. It would also be very useful to have measurements on more distant relatives, such as uncles and aunts, giving the simplified theoretical correlation of $\frac{1}{4}$, and first cousins giving $\frac{1}{8}$. Studies on twins, too, can contribute much useful information; and, in the study of normal traits, in which any pairs can be used, the supply of human twins is relatively ample. The complications of environmental reactions could be sorted out to some extent, always remembering that the balance sheet of heredity and environment is different, given different circumstances, and that relatives tend to share a common environment, the effects of which may be mistaken for a common heredity.

4. General Intelligence and Mental Deficiency

General intelligence, as estimated on standard intelligence-test scales, provides a series of measurements on which resemblances between relatives can be assessed. The literature is very large, but in one respect the data are not as satisfactory as they would be with corresponding physical measurements. A prime difficulty is that of testing adults. Intelligence scales work well with children of school age, who are broadly receiving sufficient instruction of the right kind to enable them to answer the questions correctly if they have attained the appropriate level of mental development. It is very different with adults, whose performance is progressively affected by their occupations and interests, and it becomes increasingly difficult to compare one subject with another. Another difficulty is that of sampling. It is relatively easy to secure good samples of schoolchildren, but unbiased samples of adults are hard to obtain. Moreover, there is a tendency to resent intelligence testing, with the possibility of refusals and a biased loss. Hence, while there is a profusion of data on sibs, as well as on twins, good data on parent-child resemblances and on those of more distant relatives are scarce. Nevertheless, the data on sibs and twins, with studies on twins reared apart and on foster children, together with other special observations, do permit the drawing of some conclusions. The sib-sib correlation is universally found to be of the order of 0.5 or a little more. However, this does not indicate almost complete hereditary determination. In the first place there is marked assortative mating. The correlation between husbands and wives is about 0.5 or rather more, so the theoretical 0.5 is considerably raised. Undoubtedly, too, environmental influences are contributing substantially. Sibs share not only genes but common influences which affect test performance; to quote one only, there is the background of culture in the home. It seems likely, however, in the view of the majority of observers, that inheritance contributes something like a half to three-quarters of the variation, and there can be little doubt that inheritance is, in the main, multifactorial.

One indication of an environmental component in the sib-sib correlations is provided by observations on cousins. If the sib-sib correlation is 0.5, provided that this were genetic the correlation between first cousins should be 0.125. Penrose (1939) found 0.28. In a similar study I found 0.21 (Roberts, 1940). Both samples were small, but the relatively high correlations may perhaps be taken as an indication of the action of environmental factors. Genes in common are reduced from $\frac{1}{4}$ to $\frac{1}{8}$ as we pass from sibs to cousins, and it seems unlikely that environmental similarities would be reduced so drastically. Work on these lines may well provide much additional information in the future.

The main medical interest in the distribution of intelligence-test scores, however, is its relation to mental deficiency. Mental deficiency cannot be equated to intelligence quotient (I.Q.), but is closely related to it. If we draw a line at, say, I.Q. 45, all subjects falling below that level are likely to require institutional care, or its equivalent in the home. As we go up the scale, an increasing proportion of subjects are able to get on in the community in simple occupations. But as far as a line of some kind can be drawn, we can say that most of those with I.Q.'s between 45 and 70 will require a measure of care and control in their own interests or in that of others. Above that level the proportion is small and falls off rapidly. For many years I have put forward the view, as have others, that a broad distinction can be drawn between two kinds of mental deficiency. On the one hand are those of low grade, primarily the idiots and imbeciles; on the other, those of high grade, essentially the feeble-minded. Low-grade defectives usually owe their condition to single accidents of development and the like. The high-grade subjects are essentially the negative tail of the distribution of intelligence in the general population, and causation is basically multiple.

Genetic influences are involved with both types of defective. With the low grades there are the known genetic entities such as phenylketonuria, epiloia and amaurotic idiocy, the cause in such instances being a single gene. It seems certain that some proportion of low-grade deficiency of no special identifiable type is also due to recessive genes, as was shown, for example, by Sjögren (1932). Other instances may be partly genetic, though in all probability non-genetic influences are considerably more important. With high-grade deficiency, speaking very broadly, genetic influences are apparent and show much evidence of being multifactorial in character.

It must be emphasized that the distinction between the two types of mental deficiency is far from absolute, especially in terms of I.Q. There is much overlapping. For example, we know that single genes, or other single causes, which normally produce severe defect can on occasion produce no more than moderate mental retardation. With phenylketonuria, a few subjects are no more than feeble-minded, and may indeed,

very exceptionally, have I.Q.'s within the normal range. The high-grade mongol is rare, but he exists. However, if the argument of this section is correct, the contribution of such instances to high-grade deficiency is relatively small, and it seems probable that the great bulk of subjects owe their condition to multiple causes, the genetic part of which is probably multifactorial. Space does not permit the full deployment of the argument, but some indications may be given.

One line of inquiry concerns the form of the frequency distribution of I.Q.'s. As long ago as 1914, Pearson & Jaederholm showed that the curve was more nearly Gaussian if those in "help classes", essentially the feeble-minded, were included in the distribution, than if they were omitted. My colleagues and I made a more detailed examination of the negative tail of the curve, based on a carefully derived sample of 3,361 children (Roberts, Norman & Griffiths, 1938; Roberts, 1950). It was found that the fit was very good down to I.Q. 45, but that below this point there was a considerable excess. The expected number was 0.7 against an observed frequency of 12.5. A reasonably Gaussian distribution is not proof of multiple causation and polygenic inheritance, but it is a necessary concomitant and an argument in its favour.

The next point is that the regressions within the high-grade group should be the same as in the general population. In our own study (Roberts, 1940), the regression of sibs on the subjects with the lowest 8% of I.Q.'s (omitting the 13 idiots and imbeciles) was 0.53, a figure almost identical with that for the sample as a whole. Penrose (1939) found, for his mentally defective subjects of I.Q. 50+, a sib regression of 0.44.

Some brief description may perhaps be given of an inquiry designed to throw some further light on the supposed distinction (Roberts, 1952). Two populations were sampled as completely as possible. The selected range of I.Q.'s was 35–60, which was intended to cover particularly the difficult overlapping part of the two distributions; 271 subjects and 562 sibs were tested. It was found that in regard to all analyses, type of frequency distribution, regressions and so on, the sample was very heterogeneous indeed. An attempt was then made to separate the subjects into the supposed two groups. It was necessarily an arbitrary procedure and involved a considerable measure of subjective judgement; nevertheless it did seem that the separation had been largely successful and that the very heterogeneous total group could be separated into two sub-groups having the expected characteristics. First of all, the frequency distribution of sibs was reasonably Gaussian for both groups. The sibs of the presumed low-grade subjects were distributed around a mean I.Q. of about 100, and the distribution was relatively normal except for a small hump at the negative tail, representing the small proportion of similarly affected sibs. The sibs of the presumed high-grade subjects, on the other hand, were centred on a mean of about 80 (as they should be), and this distribution, too, was reasonably Gaussian. Turning to regressions, the sibs of the presumed high-grade subjects gave a regression of 0.51, very close to expectation. With the low-grade group the regression was not significantly different from zero.

In view of the subjective nature of the division it was very desirable to apply other criteria, independent of the process of selection. These tests, too, favoured the hypothesis. Assessment by father's occupation and by classification of the home showed that the low-grade group scored markedly higher than did the high-grade group. The fertility of the mothers also was according to expectation. The mothers of subjects in the imbecile group had a mean number of pregnancies of 3.06 against 4.30 for the mothers of subjects in the high-grade group. Thus, taking the evidence as a whole, it does seem reasonable to conclude that the bulk of high-grade mental deficiency is multiple in causation and that the genetic element in causation is multifactorial.

5. Arterial Pressures and Essential Hypertension

There are few human diseases which are specified by a measurement. The outstanding example is benign essential hypertension. Clinicians have long been convinced that this is a disease entity, though attempts to fix levels of pressure which separate normal from abnormal have been fraught with difficulty. Many different cut-off points have been proposed, though, curiously, little attention has been paid to the effect of increasing age, for on the average, though only on the average, arterial pressure rises continuously throughout life. Adopting any of the favoured cut-off points, it is apparent that the genetic element is strong. Weitz (1923) was probably the first observer to point out that the ratio of affected to normal subjects suggested the action of a single dominant gene. Another study leading to the same conclusion was that of Allan (1933). An alternative explanation was put forward by Hamilton, Pickering, Roberts & Sowry (1954a, 1954b, 1954c). They suggested, in briefest essentials, that level of arterial pressure is a graded character like stature, and that benign essential hypertension represents the positive tail of the frequency curve. The first and main consideration which led them to this hypothesis was the observation that relatives of hypertensive subjects had pressures which, on the average, were raised at all ages, even down to the age-group 10–19 years. Moreover, when the deviations from average pressures at the various ages were measured in units of the standard deviation, the departure from expectation was approximately the same at all ages. They found that the resemblance between subjects and their first-degree relatives (parents, sibs and children) could be expressed by a regression coefficient of a little more than 0.2. This measure of resemblance appeared to be the same for subjects of all levels of arterial pressure from the lowest to the highest, though perhaps excluding a very small number of the highest pressures of all in the hypertensive group. Moreover, amongst those who would be considered hypertensive by any standards, there were degrees of hypertension which were duly reflected in the relatives. Subjects with higher pressures had relatives whose pressures were higher on the average than the relatives of those, still formally hypertensive, who had lower pressures.

A comparison with the results of Barnes & Browne (1945) on young women is illuminating. They classified those whose pressures exceeded 120 mm. Hg (systolic) and 80 mm. Hg (diastolic) before conception, or during the first 20 weeks of pregnancy, as suffering from essential hypertension of pregnancy. The arterial pressures of the relatives were strikingly similar to the distributions found by Hamilton *et al.* (1954a, 1954b, 1954c) for relatives of subjects with essential hypertension, who had diastolic pressures of 100+ mm. Hg. The relatives of pregnant women with toxaemia of pregnancy had lower pressures (Roberts, 1958). This is another indication that, in the absence of a specific cause, a moderate average rise in young people is equivalent genetically to a much bigger one in older subjects.

For the control subjects and their relatives, the data of Hamilton *et al.* (1954a, 1954b, 1954c) were not very ample or satisfactory, but this was remedied by Miall & Oldham (1955, 1957) who, in a very perfect sample of subjects and relatives drawn from the general population, found a closely similar regression. In a total of 623 subjects and 2,245 first-degree relatives, the systolic regression was 0.24; the relationship was independent of the blood pressures of the subjects, applying equally to those whose blood pressures were below the average and those whose pressures were above it.

The controversy between those who favour, respectively, the qualitative and the quantitative view has recently become lively (Morrison & Morris, 1959; Platt, 1959; Oldham, Pickering, Roberts & Sowry, 1960). Platt pointed out that, if relatives of ages 45–59 years of hypertensive subjects aged 45–59 were alone considered, the frequency distribution of arterial pressures, as shown in several of the recent investigations, was distinctly bimodal, suggesting a mixture of two different populations. This was countered by the suggestion that the bimodality might be partly due to digit preference and also to the desire of the observer, deliberate in some instances, perhaps unconscious in others, to assign pressures definitely either to the "normal" or to the "hypertensive" range. Moreover, bimodality, though a point in favour of a qualitative difference, is not conclusive evidence. It could, for example, be explained by a general tendency for systolic pressures to increase rapidly over the range of, say, 140–170 mm. Hg, and correspondingly for diastolic pressures.

The genetic interest of the problem is that it squarely contrasts a single gene hypothesis and a multifactorial hypothesis. On the qualitative view heritability is, as already mentioned, very high. The presumed single gene must have a number of properties. Thus, it is a dominant gene, with no evidence that it produces a larger effect in the homozygote. This is, of course, quite possible, though perhaps unexpected. It must be an extremely common gene. Søbye (1948) in his thorough survey, adopting the cut-off points of 160 mm. Hg (systolic) and 100 mm. Hg (diastolic), pushes the argument to its logical conclusion and estimates the frequency of the gene at 30–40%. There is no room for any appreciable lack of penetrance. Something like full hereditary determination must be assumed. The estimate of the degree of heritability is so high that it is not easy to allow for the fact that errors of measurement and, much more important, fluctuations with time, must reduce the observed degree of resemblance appreciably. It is not easy to explain why so harmful a gene could have become so common in the population. It has been argued that its effect is manifested after reproductive life is largely over. Theoretically it might be maintained that a gene producing its effect after reproductive life might become common, for natural selection is not operating against it. It might even be argued, perhaps, that those whose forbears do not live too long are at a selective advantage. But in fact the diseases and mortality of post-reproductive life show little, if any, evidence of simple Mendelian ratios. Nor, apparently, are these observed in experimental animals.

On the quantitative, and multifactorial, view the degree of heritability in level of arterial pressure generally (including essential hypertension) is very modest, being represented by a regression of no more than 0.23 or so, though it must be remembered that errors of measurement and individual fluctuations have lowered the true measure of resemblance. On this view, the extremely high degree of apparent heritability on the qualitative hypothesis is an artifact; it is the inevitable consequence of selecting a cut-off point between normality and abnormality which is close to the level attained by nearly half the members of the population who live long enough.

6. The Genetic Component in the Causation of Common Diseases

Many common diseases show some evidence of a measure of genetic determination. The incidence amongst relatives is found to be somewhat higher than in the general population. To quote a single example, Doll & Buch (1950) found that 11.5% of brothers of men with duodenal ulceration were similarly affected, against an expected figure for the general population (for comparable ages) of 5.5%. In instances such as these it is possible to invoke a dominant, or intermediate, gene of low penetrance. It is true that the presence or absence of disease is an all-or-none phenomenon, but there could be a threshold, and it seems more plausible to suppose that, as a general rule, degrees of underlying genetic resistance or susceptibility will ultimately be found to be multifactorial. This is what Grüneberg (1951) has called "quasi-continuous variation".

To end on a highly speculative note, with some human cancers there is evidence of a slight degree of resemblance between relatives, this being specific for particular cancers and not general for cancers of all types. Yet in experimental animals many strains have been developed which show high incidences, clearly genetically determined, of particular kinds of neoplasm. It may be, perhaps, that the genetic element is multifactorial, with many genes involved. In a more or less randomly breeding species like man, a large part of the genetic variation is concealed. Mather (1960) points out that the proportion of the variation which is concealed is $(k-1)/k$, where k is the number of gene pairs. Thus, with 10 pairs, 90% of the variation is concealed; with 100 pairs no less than 99%. In the development of highly inbred experimental strains, this variation is unscrambled, and so it may be that combinations of genes making for particular susceptibilities become concentrated and stabilized, something that does not happen in a randomly breeding population.

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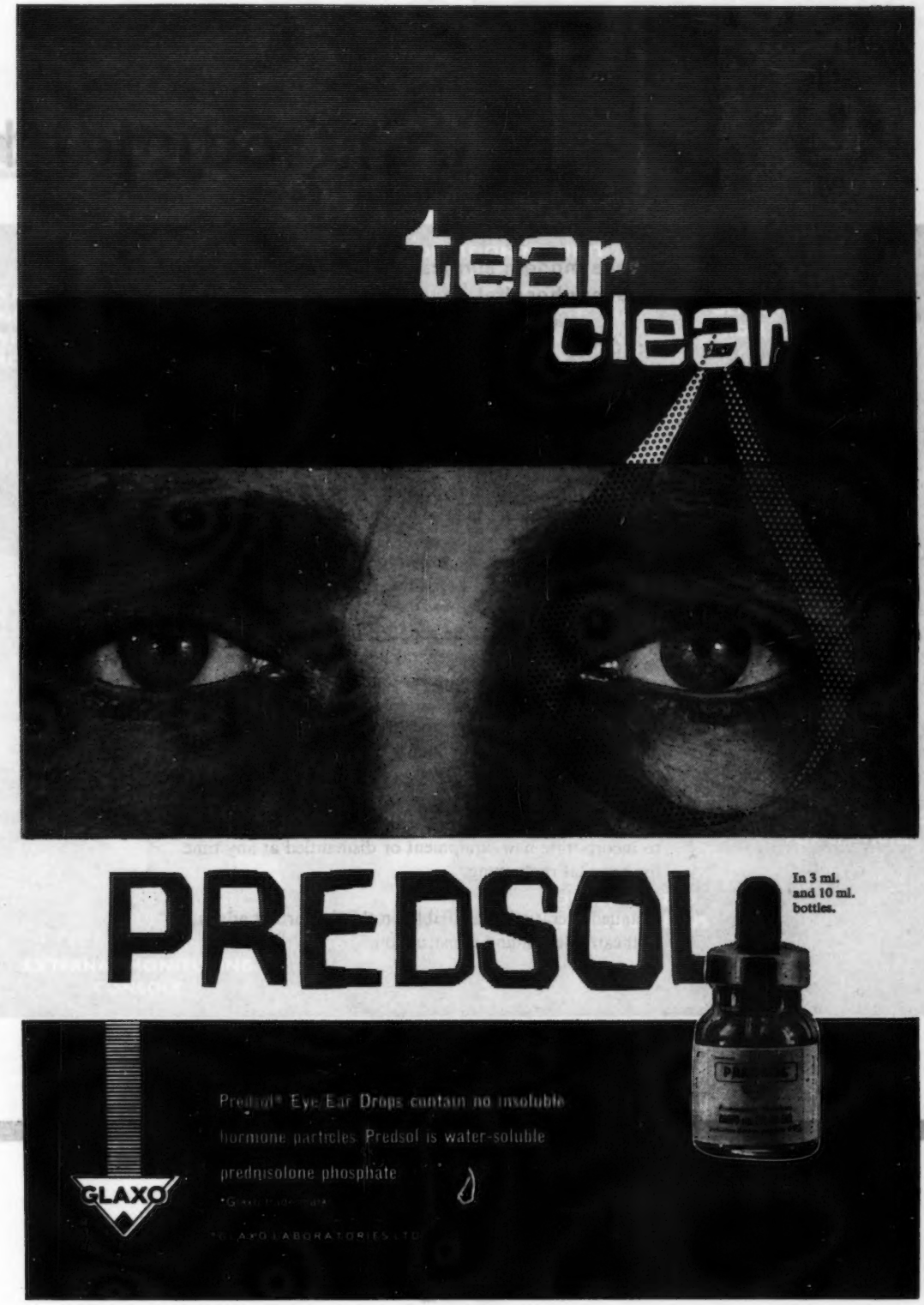
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QUANTITATIVE GENETICS OF FINGER-PRINT PATTERNS

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1 The quantitative approach
2 The method of ridge-counting
3 The use of familial correlations
4 Distribution of total ridge-count in the population
5 Inheritance of total ridge-count
6 Diversity of ridge-counts from finger to finger
7 Finger ridge-count in mongolism
References

The dermal ridges on finger balls (tips) are arranged in the form of patterns. These patterns, studied from impressions of the ridged skin, can be classified into three main types: arches, loops and whorls. This classification is based on the number of triradii present. Thus, there is no triradius in a simple arch, one in a loop and, typically, two triradii in a whorl (see fig. 1).[1]

For many years, evidence has accumulated which suggests that finger-print patterns are determined by heredity. In particular, the finger-print patterns of monozygotic twin pairs, although never identical, are very similar, while those of dizygotic pairs generally show considerably less agreement. Yet genetic investigations on pattern types have yielded indecisive results, and we still do not know how they are inherited (see, e.g., Elderton, 1920; Grüneberg, 1928; Böhmer & Harren, 1939; Essen-Möller, 1941). Qualitative analyses of other features of finger-print patterns, such as form (Bonnevie, 1924; Mueller, 1931; Geipel, 1937) and direction (Bonnevie, 1924; Newman, 1930), have likewise led to inconclusive results. (An account of genetic researches on finger-print patterns is given by Cummins & Midlo, 1943.) A new approach to the genetics of digital ridge-patterns was, therefore, needed.

[1] A triradius is the junction of three regions each containing systems of ridges which are approximately parallel in small fields of these regions (Penrose, 1954).—Ed.

1. The Quantitative Approach

Although Galton was the first investigator to attempt the measurement of hereditary likeness in finger-print patterns, it is to Bonnevie that we owe an efficient method of quantifying the dermal ridge-patterns for genetic purposes. More than thirty years ago she developed the method of ridge-counting which had first been used by Galton (1895) as a subclassification of loops. Henry (1900) incorporated the method in his system of finger-print classification for personal identification and gave rules for ridge-counting. Bonnevie (1924) extended the technique for application to all types of pattern.

Bonnevie's object in using the ridge-count as a basis for genetic studies was to overcome certain difficulties inherent in work on finger-print patterns. For instance, classification is complicated by the occurrence of a complete series of transitions between the three major pattern types while, frequently, fingers of the same individual show different patterns. By using ridge-counts Bonnevie was able to obtain a single value for an individual, consisting of a quantitative assessment of the patterns on the ten fingers.

FIG. 1. Examples of the Three Basic Types of Finger-Print Pattern

Fig. 1A — A: arch (no triradius)

Fig. 1B — B: loop (one triradius)

Fig. 1C — C: whorl (two triradii)

There is no line of count on A, and the score is 0.
In B the triradius is on the left, at the junction of three ridge systems. It is formed of three meeting ridges. The white line joining the point of the triradius to the centre of the loop illustrates the method of ridge-counting. The number of ridges cutting the line is 13.
The white lines on C are the two lines of count, one from each triradius to the centre of the whorl. The ridge-count on the left of the pattern is 17, that on the right is 8.

2. The Method of Ridge-Counting

With a low-power binocular microscope the ridge-count of a pattern can easily be determined from a print obtained by rolling the finger on the recording surface. The number of ridges which cut or touch a straight line from the triradius to the core or centre of the pattern is counted. The triradius is not included in the count, nor is the final ridge when it forms the centre. Ridges which run close to the line without meeting it are excluded, but two ridges resulting from a bifurcation are both counted. There is only one line of count for a loop, but in the case of whorls there are two, one from each triradius to the centre or to the corresponding cores. (A symmetrical whorl has a single centre, while, in the double-loop type of whorl, there are two cores.) For recent work on genetics only the higher count has been used in the case of whorls; Bonnevie, however, for some time, made use of both counts. In simple arches with no triradius, there is no count, and for this type of pattern the score is 0. The method of ridge-counting as applied to a loop is shown in fig. 1B, and to other patterns in fig. 1A and fig. 1C.

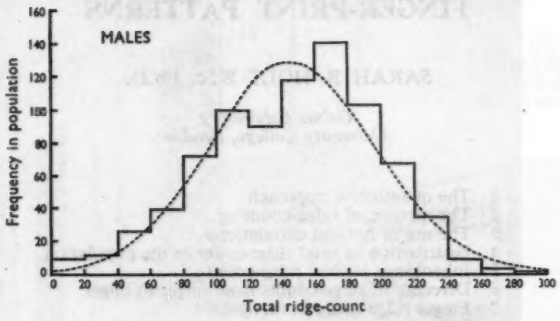
The ridge-count provides an estimate of pattern size. Unlike direct measurement of the distance from triradius to core, it is independent of age. Though it is a somewhat arbitrary measure, the ridge-count has other advantages. It is an objective feature of patterns and provides a good basis for statistical analyses, particularly as the counts on the ten fingers of an individual can be summed to give a single value, the total ridge-count. Pattern size and pattern type are not synonymous, yet the ridge-count does give some indication of the type of pattern. Thus, counts of 0 denote arches, whereas low counts are usually associated with small loops, though small whorls are sometimes found; high counts indicate either large loops or whorls.

3. The Use of Familial Correlations

The correlation coefficient, invented by Galton, was much used by Karl Pearson and his associates for the measurement of hereditary likeness between human relatives. The method of correlation as a means of determining the mode of inheritance of a character was useless, however, before Fisher (1918) published his paper on the correlation between relatives, on the supposition of Mendelian inheritance. Fisher's mathematical theory provided the means for interpreting the correlations found between various types of relative in terms of Mendelian genetics.

Bonnevie (1924), using a "quantitative value" based on the ridge-count, found the correlations between monozygotic twins (0.92 ± 0.04), dizygotic twins (0.54 ± 0.08) and sibs (0.60 ± 0.12). Her samples, however, were small, but some years later Newman (1930) repeated her calculations for a modified form of the quantitative value on a larger sample of twins. His estimates were similar: 0.95 ± 0.01 for monozygotic and 0.46 ± 0.08 for dizygotic twins. From this time ridge-counting was used to a considerable extent in studies of twins, yet it was not until comparatively recently that the correlations between parents and children were studied, nor had the correlation between sibs been calculated, apart from Bonnevie's estimate for the quantitative value. For the determination of methods of inheritance, information from all these types of relationship is needed. The total ridge-count has been found to be the most consistent and reliable measurement for familial investigations. Statistical analyses of total ridge-count have included parent-child and sib-pair correlations, and the mathematical theories of Fisher (1918) and Penrose (1949) have been used to find the mode of inheritance.

FIG. 2. Total Ridge-Count Distribution (with Grouping Interval 20) for 825 British Males, and the Calculated Normal Curve with the same Mean and Standard Deviation



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4. Distribution of Total Ridge-Count in the Population

The values for total ridge-count found in the British population range from 0 to about 300. The curve of frequency distribution for 825 males from a population sample (Holt, 1955) is non-Gaussian, being negatively skew and flattened (fig. 2). The mean ridge-count is 144.98 with a standard deviation of 51.08 ridges. The curve of distribution for 825 females in the sample (fig. 3) is also negatively skew and flattened, but the mean is significantly lower than in males—127.23 ridges—while the standard deviation is 52.51.

FIG. 3. Total Ridge-Count Distribution (with Grouping Interval 20) for 825 British Females, and the Calculated Normal Curve with the same Mean and Standard Deviation

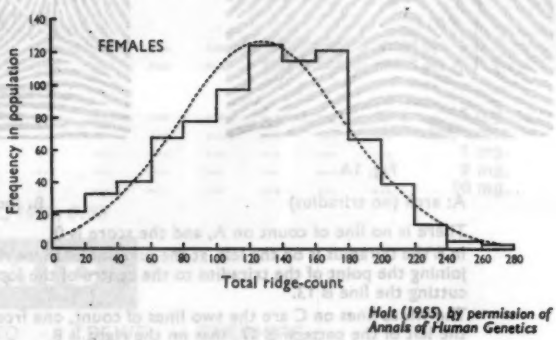

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TABLE I. Correlations Between Relatives for Total Ridge-Count

Type of relationship	Correlation coefficient	Number of pairs used
Parent-child	0.48	810 (from 200 families)
Mother-child	0.48 ± 0.04	405 (from 200 families)
Father-child	0.49 ± 0.04	405 (from 200 families)
Parent-parent	0.05 ± 0.07	200 (from 200 families)
Mid-parent-child	0.66 ± 0.03	405 (from 200 families)
Sib-sib	0.50 ± 0.04	642 (from 290 sibships)
Monozygotic twin-twin	0.95 ± 0.01	80
Dizygotic twin-twin	0.49 ± 0.08	92

5. Inheritance of Total Ridge-Count

Familial correlations for total ridge-count are given in Table I. The parent-child and sib-sib correlation coefficients are very nearly 0.5 (Holt, 1952, 1956, 1957a). According to Fisher, this is the value to be expected in both cases when inheritance is due to perfectly additive genes, and dominance is absent. In this connexion, it should be noted that, while total ridge-count is in fact a discontinuous character (i.e., one where classes can only be expressed in whole numbers—numbers of ridges), it behaves in a similar manner to a continuously varying one (i.e., where the gradations between classes are limited only by methods of measurement). Assortative mating increases all measurements of hereditary likeness, so that it is necessary to know the inter-parental correlation for the character in question. It will be seen that there is no significant parent-parent correlation for total ridge-count. The value (0.05 ± 0.07) is less than its standard error, showing that mating is random as far as pattern size is concerned.

The correlation between monozygotic twins is very high: 0.95 ± 0.01 for a sample of British twins (Holt, 1957b). Similar values for total ridge-count have been obtained by other workers—compare the correlation coefficients for a large French series of twins (Lamy, Frézal, de Grouchy & Kelley, 1957). In contrast, the correlation between dizygotic twins is of the same order as that between ordinary sibs. Small environmental effects amounting to about 5% of the total variability are suggested by the value of the monozygotic twin correlation. The similar values of mother-child and father-child correlation coefficients provide further evidence that the effect of environment on ridge-count cannot be large. Any effect must be a maternal one. The dermal ridges are differentiated in the third and fourth months of foetal life, and differentiation is complete five months before birth. After that time, neither the detailed structure of the ridges nor the configurations formed by them change, except in size, throughout life. It is, therefore, only in the uterus that environment can operate on the ridges and their arrangements.

The relationship between parents and children can be investigated in another way. The correlation between the average measurement for the two parents (Galton's mid-parental value (Galton, 1889)) and the children can be estimated. Penrose (1949) has shown that in this case the expected correlation coefficient is $\frac{1}{\sqrt{2}}$, or 0.71, when additive genes with independent effect are present. The estimated mid-parent-child correlation for total ridge-count was found to be 0.66 ± 0.03, a value agreeing well with the theoretical expectation. The mid-parent-child correlation for 199 families with 404 children is given in Table II. In each column, the cell with the number of children having total ridge-counts in the same group as the average value for their parents has been outlined. Omitting the first column, where there is only one entry, the outlined cell contains the maximum number of entries in the particular column. These cells lie on a straight (diagonal) line. This suggests, and more elaborate tests confirm, that the regression of total ridge-count of children on mid-parent value is linear. Penrose postulated that, with suitable data, linear regression of children on the average value for parents suggests perfectly additive genes. The calculated regression coefficient for the data in Table II is 0.9, a good approximation to the theoretical value of 1.0 for a gene effect without dominance.

TABLE II. Mid-Parent-Child Correlation (Data from 199 Families with 404 Children)

Total ridge-count of child	Mid-parent ridge-count							Total
	0–39.5	40–79.5	80–119.5	120–159.5	160–199.5	200–239.5	240–279.5	
240–279				1	3	1		5
200–239			1	9	13	14		37
160–199		4	7	44	33	8		96
120–159		4	31	66	17	2		120
80–119		7	40	28	8			83
40–79	1	11	22	6				40
0–39		9	12	1	1			23
Total	1	35	113	155	75	25		404

We may conclude, therefore, first, that total ridge-count is an inherited metrical character; secondly, that a number of perfectly additive genes are concerned; and thirdly, that prenatal environment plays a comparatively small part. The negative skewness of the frequency distributions in populations suggests that the number of genes having appreciable effect is fairly small. Presumably the effect of natural selection on pattern size is extremely slight, and it is for this reason that the mathematical theory holds almost exactly.

6. Diversity of Ridge-Counts from Finger to Finger

There are strong indications that another feature of ridge-count distribution is under genetic control, namely, the diversity of counts from finger to finger. Even two individuals with the same total ridge-count can have quite different ridge-counts on their separate fingers. Thus, the ridge-counts on all the fingers of one may be nearly the same, while on the other some fingers may have low counts and the remainder high ones. A suitable measure of the diversity is the sum of squares, S^2, of deviations of the ten separate digital counts from their mean (Holt, 1958). The quotient $\left(\frac{S}{\sqrt{10}}\right)$ is more symmetrically distributed than S^2 and has been used as an index of diversity. Familial correlations for this index have been calculated (Holt, 1960). The values obtained for parents and children, for sibs and for dizygotic twins, are much lower than the corresponding correlations for total ridge-count; none is significantly different from 0.25. The estimated correlation between monozygotic twins is also lower, 0.73 ± 0.06, so evidently environmental influences during early prenatal development have a considerable effect on this character.

7. Finger Ridge-Count in Mongolism

The study of distortions in dermal ridge arrangements produced by chromosome aberrations is likely to extend our knowledge of the genetics of dermal ridges. Cummins (1939) showed that in mongolism, where there is an extra autosome, the finger-print patterns as well as the palmar configurations are distorted, though to a lesser extent. Finger-print patterns of mongols show less variation, and the mean finger ridge-counts differ from those of the general population (Holt, 1951). Moreover, the frequency distribution for total ridge-count has a markedly smaller variance in mongols. Penrose (1960) has pointed out that the results of quantitative researches on the position of the axial triradius, *t*, (see Penrose, 1954) " . . . suggest that genes which govern the position of the *t* triradius may be located on the same chromosome which produces mongolism when it is present in triplicate". It is possible that genes responsible for pattern size, as measured by total ridge-count, may also be situated on this chromosome. Work is at present in progress at the Galton Laboratory to test this hypothesis.

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THE INHERITANCE OF CONGENITAL PYLORIC STENOSIS

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1 Pathology
2 Clinical features
3 Environmental aetiology
  a Evidence from studies of twins
  b Evidence for environmental factors
4 Genetic aetiology
5 Conclusions
References

Congenital pyloric stenosis is in Britain the commonest disorder in children requiring surgical intervention. The study of its aetiology has much in common with the study of the aetiology of many other common disorders of infancy. It may be shown that environmental as well as genetic factors are important in causing the disorder; but the nature of the environmental factors is not yet clearly known, and the mode of inheritance is not simple. New data, however, are becoming available from which to formulate hypotheses on the mode of inheritance. Until a generation ago, the mortality was high. Figures from The Hospital for Sick Children, London, are shown in Table I (Carter & Powell, 1954). Because of this high mortality, although it was possible to estimate the incidence of the condition in the brothers and sisters of index patients, it was not possible in their children. For the same reason, one could not expect to find that the parents were often affected. However, in 1912, Rammstedt, almost by accident, hit upon the quick and relatively atraumatic simplification of Fredet's operation. Rammstedt's operation is still the treatment of choice. This operation was introduced into Britain in 1917, and led, together with improvements in anaesthetic technique, to a rapid fall in case mortality. From 1923 onwards, considerable numbers of children with pyloric stenosis survived, and these are now founding families which will provide evidence on the proportion of children, and in turn of grandchildren, who also develop the condition.

TABLE I. Treatment and Number of Survivors of Children with Pyloric Stenosis from The Hospital for Sick Children, London, 1918–25

Years	Rammstedt's operation		Medical treatment		Total	
	Survivors	Dead	Survivors	Dead	Survivors	Dead
1918–19	6	14	1	6	7	20
1920–21	25	26	4	14	29	40
1922–23	55	18	0	6	55	24
1924–25	72	15	0	1	72	16

1. Pathology

The essential pathology of congenital pyloric stenosis is not certainly established, and so provides little information on the causes of the disease. The muscle fibres, particularly of the circular muscle layer of the pylorus, are hypertrophied, but it is not certain whether they are also increased in number. The ganglion cells of the intramuscular plexus and of the subserosal plexus undergo degeneration during the course of the illness, but this is probably a secondary phenomenon.

2. Clinical Features

The question arises: is the tumour congenital? Certain clinical features of the disorder have a bearing on its aetiology. It is not certain that the hypertrophy is truly congenital in the sense of being present at birth. In Sweden, where they have much experience of the radiological appearances of the disorder, it has been found that infants who have later developed pyloric stenosis have shown no abnormal radiological appearances shortly after birth (Wallgren, 1946). However, symptoms not uncommonly start in the first week of life, and pyloric tumours have been found at operation as early as the fifth day of life. There is no satisfactory report on a pyloric tumour's being found in a still-born child. In premature infants, however, in whom the condition occurs with the same frequency as in any infants born at full term, symptoms often develop before the child's expected date of birth. It would appear, therefore, that a short period of extra-uterine life is needed for the tumour to develop sufficiently to cause symptoms. The tumour probably continues to develop during the first few months of life, since the later the age at operation, the larger the tumour tends to be (McKeown, MacMahon & Record, 1951a). It is noteworthy that the tumour may be present without causing symptoms at any time (Carter & Powell, 1954).

The ratio of male to female patients varies in different series from 4:1 to 5:1.

Population Frequency

The incidence of confirmed cases in England and Scotland is about 3 per 1,000 live-births (see Laron & Horne, 1957; Smith, 1960). The condition has a similar incidence in Sweden where, however, it appears to have fallen from 4 per 1,000 to 2 per 1,000 over the last decade (Wallgren, 1960). The incidence is probably of this order all over northern Europe. It appears to be less common in American "whites" and southern Europeans, less common still in negroes, whether in America (Laron & Horne, 1957) or Africa, and rare in Asiatics. It is improbable that all these differences are merely due to differences in completeness of ascertainment. Allowing for the sex ratio of 5:1, the incidence in Britain is about 1 per 200 male live-births, and 1 per 1,000 female live-births.

3. Environmental Aetiology

a. Evidence from Studies of Twins

No satisfactory study of congenital pyloric stenosis in twins has yet been made. There are several individual reports, and three small series (see Metrakos, 1953) in which the type of

twinning was accurately established, and one large-scale study in which no attempt was made to establish the type of twinning (MacMahon & McKeown, 1955). Together, these studies suggest that the proportion of monozygous, or "identical", co-twins of patients also affected is higher than that of dizygous, or "fraternal", co-twins also affected, indicating that genetic factors are important in aetiology. These studies also suggest, however, that as many as 50% of monozygous co-twins of patients are unaffected, and this difference between individuals with the same genotype must be attributed to the effects of environmental differences.

b. Evidence for Environmental Factors

There are three clues to environmental factors, which may be important in the aetiology of congenital pyloric stenosis. The first is that the condition may be more common in first-born children among patients where the onset of symptoms is in the third week or later. The second is that the onset of symptoms is later in children born in hospital than in those born at home (McKeown, MacMahon & Record, 1952). The third is that the onset of symptoms is later in children fed four-hourly than in those fed three-hourly (Gerrard, Waterhouse & Maurice, 1955). None of these effects is marked, but together they suggest that skill in feeding the baby may postpone the onset of symptoms. Since clinical manifestations of the disorder do not develop after the end of the fourth month, such skill may occasionally prevent the development of symptoms altogether.

4. Genetic Aetiology

An early study from London (Cockayne & Penrose, 1943), and a more recent study from Birmingham (McKeown, MacMahon & Record, 1951b), established that the risk to brothers and sisters of patients with pyloric stenosis was much increased over the risk in the general population. The authors of the former study tentatively suggested that the genetic predisposition might be due to a recessive mutant gene. The authors of the latter study stressed the probable importance of environmental factors.

The first evidence on the proportion of affected children of patients with pyloric stenosis came from a small London series collected by Carter & Powell in 1954. They attempted to trace patients treated by Rammstedt's operation, the diagnosis being confirmed at operation in two London hospitals between 1920 and 1930. A small series was reported from Denmark (Nielsen, 1954) later the same year, which was similar except that the diagnosis was inevitably less exact, since the affected children, both in the parental and filial generation, were treated medically and not surgically. These two small series indicated that the risk to sons and daughters of men and women who had pyloric stenosis in infancy is probably of the same order as the risk to brothers and sisters.

In the last few years, I have had the opportunity of adding extensively to the London series. A largely successful attempt has been made to trace all the 774 men and 145 women who had been treated at The Hospital for Sick Children, London, between 1920 and 1940, with confirmation of the diagnosis by Rammstedt's operation, and who had survived operation and the early post-operative period. By various means it has been possible to trace 562 surviving males and 96 surviving females, this being 72% and 67% respectively of the series. The reasons for failure in attempts to trace these patients were not such as were likely to introduce bias from the view-point of genetic analysis. The reasons included death, migration, adoption, temporary residence overseas on National Service, and instances where the name and address on the original hospital records were insufficient. So far, 174 of the male patients, and 48 of the female patients, have had at least one child. These have been visited and have provided more detailed information than has previously been available on the proportion of sons and daughters of patients with congenital pyloric stenosis who are also affected; they have also provided useful supplementary information on the risk to brothers and sisters of patients.

Strict criteria were adopted for classing a relative as affected. In most instances, the diagnosis was based on a hospital record of a Rammstedt's operation in which a tumour was found at operation. Cases with suggestive symptoms that were treated medically were not classed as affected, unless, in addition, there was a record of a pyloric tumour's being felt by an experienced medical practitioner. The information on this basis on the sons and daughters and brothers and sisters is set out in Table II for the male index patients, and Table III for the female index patients. Because of the criteria adopted, these figures probably somewhat under-estimate the total numbers clinically affected, by the exclusion of a few mild cases and also of a few severe cases causing the child's death before the diagnosis was confirmed.

TABLE II. Incidence of Pyloric Stenosis in Relatives of 174 Male Index Patients

	Brothers	Sons	Sisters	Daughters
Affected . .	5	11	5	2
Total . . .	156	162	165	161
Percentage affected	3.2	6.8	3.0	1.2

TABLE III. Incidence of Pyloric Stenosis in Relatives of 48 Female Index Patients

	Brothers	Sons	Sisters	Daughters
Affected . .	5	9	1	4
Total . . .	38	44	40	36
Percentage affected	13.2	20.5	2.5	11.1

Though the numbers affected are small, the following conclusions may be drawn from the data shown in Tables II and III.

i. The proportion of sons and daughters of index patients affected, 6.5 (± 1.2) %, is almost certainly not less than, and probably more than, the proportion of brothers and sisters affected, 4.0 (± 1.0) %. The proportion among the children is, in fact, higher in every comparison except that between daughters and sisters of male index patients; this apparent exception may well be due to chance. This finding makes it unlikely that recessive genetic determination, which typically gives higher risks in sibs than in children, is important in pyloric stenosis.

ii. There are 11 examples of fathers who had affected sons. This finding makes it unlikely that the marked preponderance of males among patients with pyloric stenosis is due to sex-linked inheritance. Men do not transmit their X chromosome to their sons.

iii. Although only one patient in six with congenital pyloric stenosis is female, the relatives of the female index patients have a correspondingly higher risk of being affected. In this series, the 48 female patients provided 19 affected first-degree relatives, which is almost as many as the 23 provided by the 174 male index patients.

iv. When compared with the incidence in the general population of the same sex, the increase in the proportion affected is rather higher in female than in male relatives of the index patients.

If one assumes that the risks to brothers and sisters, and to sons and daughters, of index patients are essentially the same, the findings may be summarized and approximately related to the incidence in the general population, as shown in Table IV.

TABLE IV. The Proportion Affected of First-Degree Relatives (Sibs and Children) of Index Patients Compared with the Proportion of all Children of the same Sex Affected

Category of relationship	Proportion affected	Approximate comparison with general population of same sex
Male relatives of male index patients	5 (±1)%	×10
Female relatives of male index patients	2 (±1)%	×20
Male relatives of female index patients	17 (±4)%	×35
Female relatives of female index patients	7 (±3)%	×70

5. Conclusions

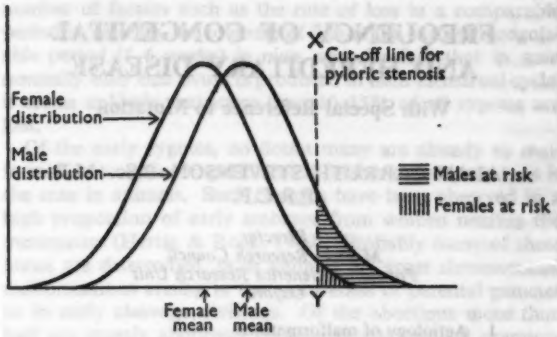
Any hypothesis of a genetic mechanism to account for these new findings in families must be tentative, particularly because of the unknown environmental factors of considerable importance which determine whether the predisposing genotype results in clinical manifestations. It is clear, however, that concentrations of affected subjects in families of the kind found are most unlikely to be due only to common family environment.

Any appreciable degree of either autosomal recessive or sex-linked recessive genetic determination is contra-indicated, as mentioned above, by the new data on the proportion of children of index patients who also have congenital pyloric stenosis.

Probably the simplest genetic model which fits the findings shown in Table IV and the evidence from twins has the following character. The predisposing genotype has two components, first a common dominant gene, and second a sex-modified multifactorial background. The evidence from monozygous twins suggests that only about half of those with this predisposing genotype develop pyloric stenosis.

The multifactorial background is assumed to be similar to that which holds for the genetic determination of stature. Both sexes show a normal distribution with similar variation about the mean, but the mean and the distribution in males is shifted well to the right of that in females. This is represented diagrammatically in fig. 1.

FIG. 1. Hypothetical Distribution of Multifactorial Genotypes Contributing to Pyloric Stenosis in Males and Females



If only those beyond a line XY are capable (if they also possess the dominant gene) of developing pyloric stenosis, a considerably higher proportion of males than of females are at risk, and affected females have genotypes more extreme than those of affected males. Further, it is a feature of multifactorial inheritance, without dominance and without assortative mating, that the first-degree relatives of exceptional individuals tend to have values distributed round a mean half-way back towards the general population mean. The first-degree relatives of the female index patients will, therefore, be distributed well to the right of the population means, though the variance will be somewhat less than in the general population; this shift to the right will bring a substantial proportion of the males and fewer, but still relatively many, female relatives into the area of risk. The first-degree relatives of the male index patients will also have a distribution shifted to the right of that of the general population, but to a lesser degree, giving a correspondingly smaller increase in the proportion at risk. In addition, this mechanism will account for the greater increase, when compared with the incidence in the general population, in the female relatives than in the male relatives of index patients. A genetic model of this kind can be made to fit closely the findings shown in Table IV, though this does not mean that it is necessarily the correct one.

My impression, on seeing these patients now that they are adult, is that the multifactorial component may be related to general body musculature. This is being investigated.

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Continued at the foot of page 254

FREQUENCY OF CONGENITAL AND HEREDITARY DISEASE

With Special Reference to Mutation

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1 Aetiology of malformations
2 Frequency of congenital malformations
3 Frequency by aetiological groups of malformations in the live born
4 Frequency of hereditary disease as opposed to malformation
a Single-gene traits
b Common severe diseases
5 Summary of frequencies in the live born
6 Mutation
7 Contribution of recurring mutations to the frequency of congenital and hereditary disease
References

The purpose of this review is to outline for medical readers some information on the frequencies of congenital and hereditary disease and to assess the contribution of recurring mutations to maintain these frequencies. In a sense, however, all disease has a hereditary element, and so the choice of which disorders or harmful traits are included in any review must be arbitrary. At one extreme, infectious diseases might be regarded as determined entirely by environment—although this could be challenged. At the other, there are many disorders determined by single genes whose expression is, as far as can be detected, quite uninfluenced by environmental factors.

For simplicity it is convenient to consider these congenital and hereditary disorders in three groups: (i) those malformations recognizable by the naked eye that have already arisen in intra-uterine life, conventionally termed congenital malformations; (ii) disorders or diseases determined by single-gene substitutions; and (iii) those where the genetic contribution to causation is more complex.

Such grouping on grounds of aetiology is irrational. There is overlapping in terms of age-groups affected and between macroscopic and microscopic anomalies. Further, estimates of frequencies should never be given without precise statement of which traits and disorders are included. There are many hundreds of traits and there is no detailed and generally accepted nomenclature and classification of congenital and hereditary disorders to which reference can be made.

There are many reasons for an increasing interest in all those disorders. As other causes of still-births and infant deaths have become fewer, the number of malformed infants, which has not diminished similarly, determine an increasing proportion of all losses. As infectious disorders are controlled, chronic degenerative disorders—which all have some familial aggregation—become of increasing relative importance as a cause of death. Not least, worries about the genetic effects of increases in man-made ionizing radiation have led to attempts, however hazardous in the light of our meagre knowledge, to estimate the consequences of rises of mutation rates in terms of malformation, disease and death in future generations.

1. Aetiology of Malformations

There is a very large literature on experimental teratology, and a symposium on congenital malformations (Ciba Foundation, 1960) is a convenient recent source of reference. In the present context we should like to know the relative importance of environmental and genetic components in aetiology. We should also like to know the precise genetic mechanisms involved in each malformation. We are very far from having the necessary understanding.

It is perhaps easiest to pick out first those which appear to be determined by single-gene substitutions. These, so far as we know, constitute only a small proportion of malformations and most, if not all, usually permit survival until live-birth.

The number of malformations clearly recognized to be entirely due to environmental causes is even smaller. Maternal rubella, toxoplasmosis and occasional accidental harm to the embryo from radiation or drugs are the known environmental causes.

There is, however, considerable evidence of variation in the frequency of some malformations. Thus there are marked geographical differences in the frequency of anencephaly (reviewed by Penrose, 1961) and some evidence for seasonal variations and those brought about by social class (Edwards, 1958). Searle (1958) has shown that in Singapore the differences in ethnic groups are striking.

INHERITANCE OF CONGENITAL PYLORIC STENOSIS

Continued from page 253

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It seems that while, on the whole, such evidence suggests predominantly environmental influence, there could be other explanations. All of these malformations having such frequency variations suggestive of environmental influences tend to recur in sibships much more commonly than would occur by chance. Again such phenomena might be attributable to recurrence of the same genotype in successive embryos or to a common intra-uterine environment.

The relationships may, however, be complex and the uterine environment may reflect past or present environment, maternal-foetal incompatibilities or the phenotype of the mother genetically determined. The best-known example of the latter is malformation of the uterus (Holmes, 1956; Stevenson, Dudgeon & McClure, 1959; Wilson & Harris, 1961). This is associated not only with a high frequency of other types of malformation in offspring, such as anencephaly, but also with malformation of the uterus and other anomalies in structures developed from the urogenital ridge. So it would appear that such mothers not only present an unfavourable uterine environment for development of the foetus but also contribute unfavourable genes to their offspring.

Almost a third of pregnancies resulting in malformed children are complicated by hydramnios and it seems likely that this represents some maternal-foetal incompatibility (Stevenson, 1960). It is of some interest to note that, in addition to its association with mongolism, hydramnios has also occurred with at least one other trisomic state (Edwards, Harnden, Cameron, Crosse & Wolff, 1960) but has not been shown to be associated with any trait attributable to a single gene.

We have little evidence as to the genetic mechanisms involved in any of these gross malformations not due to the less common simple single-gene traits or to chromosomal aberrations. It would appear that, in most, the determining genotypes are complex. They may result from the rare—more or less chance—coming together of combinations of genes which in other combinations are harmless. Malformations may be associated with genotypes which are relatively common but liable to determine developmental error only under stresses which would not cause malformations in other genotypes.

A number of these malformations, notably the Klippel-Fiel syndromes, fit Grüneberg's concept of quasi-continuous variation (Grüneberg, 1952). There is little to suggest—except possibly in a few instances advanced by Schull (1958)—that they represent a situation of homozygosity at too many loci, with a reduction of the number of alternative pathways in development as might be suggested by the theories of Lerner (1954) and Waddington (1957).

The important point is, however, that in any given combination of genes determining a harmful expression of genotype many or conceivably all of the genes involved may in other combinations be harmless or at most very much less harmful. We do not know and have no means of knowing to what extent the frequency of these genes is being maintained by mutation or other mechanisms. Nor do we know the frequency of such individual genes in populations.

2. Frequency of Congenital Malformations

Live-born children are the survivors of a rather rigorous selection in utero. In Britain, of all pregnancies which continue longer than four or five weeks, 12–15% end as abortions before the end of the 27th week, and rather more than 2% end as still-births.

We have no direct information about the proportion of zygotes lost earlier than the fourth week but, considering a number of factors such as the rate of loss in a comparable period in animals, the low rate of loss at the earliest recognizable period (4–6 weeks) in man, and the fact that in man normally only one ovum is produced in each menstrual cycle, it seems unlikely that more than 10–15% of all zygotes are lost.

Of the early zygotes, no doubt many are already so malformed as to be incompatible with further development, as is the case in animals. Such changes have been observed in a high proportion of early embryos from women nearing the menopause (Hertig & Rock, 1949). Probably many of these losses are determined, as in animals, by gross chromosomal malformations arising in the maturation of parental gametes or in early cleavage divisions. Of the abortions more than half are grossly abnormal on relatively superficial examination (Mall, 1917; Stevenson *et al.* 1959).

There are good data on malformation in still-born infants in whole populations (as distinct from hospital births), from Birmingham (McKeown & Record, 1960), from the Registrar General for Scotland where the cause of still-birth is registered, and from Belfast (Stevenson, 1959; Stevenson & Warnock, 1959). It seems clear that over 20% of still-born infants are grossly malformed in the United Kingdom, where the prevailing still-birth rate is about 23 per 1,000 births.

From these figures, therefore, it would seem that live-born children in Britain represent perhaps 70% of all zygotes and that of the 30% who failed to survive in utero at any stage of development at least half were malformed. There is much overlapping between the severe types of malformations which determine still-birth and those which determine early death in foetuses which survive to be born alive.

3. Frequency by Aetiological Groups of Malformations in the Live Born

We may take a round figure of 25 per 1,000 as the minimum frequency of all children born with a malformation detectable by the naked eye, whether or not the anomaly is detected or detectable at birth. The data of McKeown & Record (1960) suggested that about this proportion had been recognized by five years of age and no doubt others would be discovered later. This figure is not very different from that used in the Report of the United Nations Scientific Committee (1958). Taking the data of Stevenson (1959), which were used in this report, it would seem that, of malformations of this type, about 5 per 1,000 births are reasonably attributable to single-gene traits. To this group can be added 2 per 1,000 for mongolism and possibly another 2 per 1,000 for other chromosomal aberrations arising either in maturation of parental gametes or during early cleavage divisions. More work and more sophisticated techniques may require changes in such estimates before long.

The single-gene traits determining malformations present before birth include a number of skin conditions such as recessive ichthyosis congenita, one type of recessive epidermolysis bullosa and at least one form of dominant tylosis. There are also a number of eye conditions where some at least of the traits are monomeric, such as congenital cataracts, and the anophthalmos-cryptophthalmos-microphthalmos group.

Local skeletal malformations like the abnormalities of the extremities are also well represented (the brachydactylies, syndactylies and ectrodactylies), as are grosser limb deformities due to single genes. The last are much less common. Generalized single-gene skeletal abnormalities also come under this heading, as do such conditions as achondroplasia (not chondrodystrophic foetalis), Marfan's syndrome, and a number of dysostoses involving the skull and the rest of the skeleton. Sex-linked stenosis of the aqueduct of Sylvius, determining hydrocephalus which may have onset at widely varying ages even within sibships, is another interesting example (Bickers & Adams, 1949; Edwards, Norman & Roberts, 1961). The dominant gene syndrome of cleft palate with pits in the lower lip is, so far as is known, the only simple syndrome associated with cleft palate which is monomeric.

If we take the "Congenital malformations" in the International Classification of Diseases (1957, section 750–759), but exclude traits such as skin blemishes, accessory nipples or accessory auricles, the frequency of recognized malformation at or shortly after birth while the mother and child are still under medical care following confinement is about 15 ± 3 per 1,000. Neel (1958) and McKeown & Record (1960) have given data enabling estimates to be made of the additional numbers likely to be recognized either at death or at nine months and five years of age respectively. From these data it would appear that the estimates of 15 per 1,000 at birth may be increased to over 20 per 1,000 by five years. Even by that age, however, all internal malformations are not recognized. Some are apparent only when functional breakdown of a defective organ leads to investigation, as in the case of polycystic kidney and other malformations of the renal tract, and certain types of congenital heart disease; and in the central nervous system, porencephaly, absent corpus callosum, and cerebral and cerebellar agenesis. Other marked malformations may not give rise to symptoms and may be discovered only as an incidental finding at autopsy as, for example, again kidney malformations and absent spleen.

Table I is derived from part 1 of the Registrar General's Statistical Review of England and Wales for 1958. It is clear that malformations present before birth contribute to mortality at all ages, although inevitably to an increasing extent with advancing age. The remainder of the malformations—rather over 15 per 1,000—are presumably determined in part by environment and in part by the more complex ill-understood types of genetic mechanisms previously mentioned.

TABLE I. Deaths from Congenital Malformations by System and by Age at Death (data derived from Registrar-General, 1958)

Deaths at ages (years)	Percentages of all deaths due to congenital malformations				
	Congenital malformations of central nervous system (750–753)*	Congenital malformations of circulatory system (754)*	Congenital malformations of digestive system (756)*	Other malformations (755 and 757–759)*	All congenital malformations
0–	7.98	7.52	4.14	3.56	23.20
1–	5.21	6.75	1.96	2.78	16.40
5–	1.47	4.18	0.96	1.91	8.52
15–	0.36	2.70	0.15	0.80	4.01
25–	0.10	0.84	0.15	0.60	1.69
45–	0.01	0.14	0.03	0.31	0.49
65+	0.01	0.01	0.03	0.03	0.07

* These numbers refer to the classification used in the International Classification of Diseases (1957).

4. Frequency of Hereditary Disease as Opposed to Malformation

Disease developing after birth may have been determined by microscopic developmental anomalies already present before birth, by such anomalies arising in the course of post-natal development, or by premature or abnormal degeneration of fully differentiated tissues. Only the last type of disorder could be called abiotrophic. As we are not concerned with such sophistications here, it is convenient to treat together all disorders with hereditary components in aetiology. These fall naturally into two groups genetically, the single-gene traits which are very numerous but individually uncommon, and a range of serious diseases where nature and nurture contribute to causation in ways which are ill understood.

a. Single-Gene Traits

Several hundred single-gene traits other than macroscopic anomalies are known. Many of them are only apparent or only cause trouble many years after birth. The classical example is Huntington's chorea, but there are other examples, many in the central nervous system, such as Friedrich's ataxia and some mid-brain degenerations. Also into this group come the autosomal muscular dystrophies. In this connexion, however, it is of interest to note that gross disturbance of creatine-creatinine metabolism has been found in a very young infant many months before muscle wasting or pseudohypertrophy could be demonstrated in sex-linked muscular dystrophy of the Duchenne type (Stevenson, 1953). Some forms of progressive recessive deafness become obvious only some years after birth and possibly the majority of deaf-mutes have more hearing at birth than later.

Some of the eye disorders determined by a single gene are abiotrophic, having onset in late childhood or adult life, as in some choroidal degenerations where ophthalmoscopic examination may be normal for many years before detectable changes occur. The proportion of blind persons in a population where the blindness is due to hereditary or congenital disease varies according to the prevalence of ophthalmia neonatorum and other infections and tropical parasitic infestation. Sorsby (1950) estimates the proportion in England and Wales as about 60% in childhood, falling to about 7% by 70 years of age as degenerative and senile causes increase. A high proportion of the congenital and hereditary groups is due to single-gene traits. On the whole, however, single-gene traits which become evident only long after birth are probably the result of actual somatic change, even if microscopic, or of the presence of chemical changes which were already present at birth.

The United Nations Scientific Committee on the Effects of Atomic Radiation (1958) estimated that about 10 per 1,000 of the live born suffer or will suffer from such harmful single-gene traits "including some traits whose effects are small". This quoted phrase is a little misleading as the traits listed are, on the whole, rather severe and, if the numerous dominant gene traits having a small visible effect had been added, the total frequency would have been about 35 per 1,000. In addition, fibrocystic disease (1.0 per 1,000) and single-gene deaf-mutism (0.3 per 1,000) are not included for reasons stated in the report. This estimate includes, however, the

traits already referred to determining macroscopic anomalies before birth and estimated as having a frequency of 5 per 1,000. This estimate of 10 per 1,000, including malformations, seems rather low, and many troublesome skin and other conditions due to single-gene traits were excluded from the Committee's figure because of their relatively minor harmful effects or because individually their frequencies were low. Only a few examples of the latter group are included in the list in Table XIV, Annex H of the United Nations Report. But the total of such disorders is large, probably contributing about 3 per 1,000. So we have, in addition to 5 per 1,000 of malformations due to single genes, about 8 per 1,000 of diseases and disorders so caused.

There is another category of disease which must be mentioned as caused by genes at a single locus, that is, rhesus incompatibility, which affects in some degree about 4 per 1,000 infants, with the rhesus gene distributions as they are in the United Kingdom. This condition is, in a sense, due to single genes whose expression is harmful to the foetus only in a particular environment.

b. Common Severe Diseases

In addition to these conditions present at birth and to the single-gene effects coming on only later there are a number of medically important disorders where the pattern of aggregation of cases in families suggests that genetic factors in aetiology are very strong. The most important of such traits are diabetes and schizophrenia, but there are a number of others where the problems of finding adequate genetical hypotheses are similar: for example, pernicious anaemia, thyroid disorders and manic-depressive psychoses. (These are the disorders listed in the United Nations Report.)

Part of the difficulty is that these disorders are not necessarily true traits in the genetic sense of being the expression of a single genotype. Most of them can probably be determined by different biochemical mechanisms and, in so far as they are genetically determined, by different gene combinations. Diabetes is known to be heterogeneous, the thyroid disorders are rapidly being broken down into more understandable entities, and no doubt this process will continue in the other disorders of this kind.

Many ingenious attempts have been made to explain family and population distributions of traits of this type on a hypothesis that they are determined by single genes, but that the expression of these genes is modified in some ways. Thus, for example, failure to observe an expected distribution of traits in families in a simple Mendelian ratio is attributed to failure of penetrance or manifestation of the trait in all those of the appropriate genotype, and this manifestation rate may be supposed to differ between the sexes. Sometimes also it is suggested that some subjects with the trait represent the homozygote and others the heterozygote.

Such attempts to fit data to a simple hypothesis are, however, hazardous and fatally easy, in that any pattern of data can be fitted to a single-gene hypothesis, by introducing one or more of such modifications. Similar suspicion must fall on genetic hypotheses which call for two specified genes to be necessary for expression of a particular phenotype, or for specific modifying genes. When several modifying genes have to be postulated, then the hypothesis becomes little different from one of multifactorial inheritance with manifestations of the trait depending on environmental factors, on a physiological threshold, or even on an arbitrary definition of what is accepted as the disease. Indeed, as Edwards (1960) recalls in an interesting paper, Mendelian segregation may readily be simulated even by simple (additive) multifactorial inheritance.

The other obstacles to accepting single-gene mechanisms as determining disorders of this type are the continued high gene frequencies in the face of rather severe selection and the high mutation rates which have to be postulated to maintain the continued high frequency of the disorders. For example, in schizophrenia the fertility of affected individuals is low relative to that prevailing in the population, because of few and late marriages and small families. Yet perhaps one in eight hundred in the population are treated for schizophrenia at some time in their life.

Although these disorders begin on an average in late adult life and therefore do not affect fertility so markedly there is still, as in diabetes, considerable reduction in relative fertility, and the trait frequency is even higher in diabetes than in schizophrenia. If the high frequencies of these traits are maintained by mutation, as is required by a simple monomeric hypothesis, then the mutation rate must be very high.

In the light of our estimates of the mutation frequency of dominant and sex-linked genes in man and of spontaneous mutation rates in mammals, it seems unlikely that the mutation rate at a locus to a harmful gene would be more than one in fifty thousand.

5. Summary of Frequencies in the Live Born

From the considerations advanced in the preceding sections, the frequency appears to be as in Table II. Such figures are not very helpful. It may be that the environmental contribution is overwhelmingly important in the first group. On the other hand, there are probably included there some simple dominant gene traits and unrecognized recessive syndromes. There is much to suggest, for example, that some cardiac interventricular septal defects and some cases of truncus communis are simply inherited.

The list of common disorders is only exemplary and is incomplete. To it could be added many other conditions, such as peptic ulceration, rheumatic fever, rheumatoid arthritis, disseminated sclerosis and the auto-immune disorders, where there are varying degrees of aggregation of cases in families.

6. Mutation

Mutation rates for man can be estimated with certain reservations for severe effect dominant genes and sex-linked

TABLE II. Frequencies in Live Born of Certain Groups of Traits which are Present at Birth or Manifest in Later Life

Groups of traits	Frequency per 1,000 births
Malformations of complex genetic and environmental aetiology	20
Malformations due to single-gene substitutions	5
Disorders associated with chromosomal aberrations	4
Single-gene traits determining disease	8
Erythroblastosis	4
Common disorders with genetic components in aetiology	10
Total	51

genes. The rationale of these estimates we owe to Haldane (1935) and Gunther & Penrose (1935). The hazards inherent in them are discussed by Penrose (1957), by Stevenson (1957) and more recently by Penrose (1961), and in these and other papers representative estimates for different traits are listed. They range from about 1 to 100 mutations per 1,000,000 gametes per generation. How far such a range of values is likely to apply to other loci, where only small effect mutations occur, is very difficult to determine. Rates of spontaneous mutation of similar types of genes appear to be similar to those for man but relatively much less information is available.

It also seems likely that the order of magnitude of mutations to harmful recessive genes in man, as in animals, is similar to those for dominant and sex-linked genes. We cannot estimate mutation rates from recessive genes in man with confidence, for even more assumptions have to be made than when estimating for dominant and sex-linked genes. We have to assume that heterozygote carriers are randomly distributed in populations, whereas there are many indications of the hazards of such an assumption, a recent one concerning phenylketonuria being afforded by the evidence of high gene frequency in populations of Irish origin in London (Carter & Woolf, 1961).[1] We also have to assume that the heterozygote carrier is neither at selective advantage nor disadvantage, although we know that both phenomena occur and that we could detect only gross differences. We have to assume no gametal selection, that is, preferential contribution to zygotes of gametes, depending on whether or not they carry the gene. Finally, we cannot allow for the relaxation of inbreeding which has been going on in most populations and is certainly now operative in all populations.

Our interest in mutation in the context of this paper is to see if it is possible to make any estimate of the relationship of the frequency of mutation to that of harmful hereditary disorders in populations. This is an enormous subject, and all that can be done here is to sketch out the problems encountered in attempting to answer such a question, and to mention the attempts which have been made.

7. Contribution of Recurring Mutations to the Frequency of Congenital and Hereditary Disease

The frequency of a gene in a population may be said to depend on three factors: (i) the number of new genes arising by mutation in germ cells; (ii) the proportion of gametes carrying these newly arisen mutations which contribute to zygotes relative to gametes not carrying the mutant gene; and (iii) the proportion again relative to a non-mutated allele which, having reached zygotes, is passed on to the next generation. In short, the factors are mutation, selection in the haploid and selection in the diploid. We can, for simplicity, ignore haploid selection at the moment, although it could prove to be important (Stevenson & Jennings, 1960).

If mutation frequency does not vary over the generations, and selection against the diploid (the embryo, foetus or adult) by lesser relative fertility whatever the cause (lesser viability at any stage, infrequent marriage, late marriage or infecundity) is also stable, then the frequency of a trait determined by a gene represents an equilibrium between mutation and selection. In the case, therefore, of harmful simple dominant gene traits, where the gene manifestation is essentially independent of environmental influences or of the rest of the genotype, it can be said that the trait frequency varies directly as mutation so long as selection against the trait remains constant.

The same holds for recessive gene traits, provided that they are recessive in the traditional sense, namely, that the homozygous expression is harmful, but the heterozygote is neither at advantage nor disadvantage relative to persons homozygous for the usual allele at the locus. Under these conditions, changes in mutation rate would be reflected sooner or later in proportional changes in trait frequency, the extremes ranging from a few generations in the case of harmful dominants to perhaps thousands of generations in the case of rare recessives, as is very clearly set out and exemplified by Penrose (1956).

In the case of recessive genes, however, the heterozygote may not be "neutral" in selective value. There may be "selection for" the heterozygote determining that the subject has a greater total effective fertility relative to the population as a whole, or selection "against the heterozygote" where relative fertility is reduced. In either case the effect on gene frequency independent of fresh mutations must be considerable. In the case of uncommon autosomal recessives, heterozygote carriers have a frequency of roughly twice the square root of the frequency of those affected with the trait, e.g., if those affected occur with a frequency of about 1 per 10,000 at birth, the heterozygote carriers would have a frequency of $2\sqrt{\frac{1}{10,000}}$, or one in one hundred. As a result very small, and in practice quite unidentifiable, variations of relative fertility of heterozygotes could be of much greater importance than mutation in varying gene and therefore trait frequency.

It seems likely that such mechanisms determine the well-known balanced polymorphisms of blood groups, and other specific distinguishing traits in man. They also seem to determine the frequency of sickle-cell disease (Allison, 1954) and probably that of thalassaemia and of various discrete enzymic differences uncovered by drug sensitivities, discussed elsewhere in this symposium. They may explain the high frequency of fibrocystic disease in Great Britain and may influence the frequency in either direction of the uncommon recessive traits. As we have seen, however, only a minority of the harmful disorders considered are predominantly determined at a single locus or by gross chromosomal aberrations. Of them we can, in the light of the foregoing, surmise that their frequency is wholly or substantially maintained by mutation.

Chromosomal aberrations are also mutations in the wider sense; and present evidence suggests that in the majority of cases, where children with mongolism or other types of chromosomal aberrations are born, the defect arose in meiotic divisions in one of the child's parents. However, a proportion, as yet we have little idea what proportion, in the various traits probably arises in mitotic divisions during early cleavage. If we assume that the mutation rate of all alleles is the same at the rhesus locus, then the frequency of erythroblastosis would be independent of mutation.

For the rest, where we suppose that the disorders result from complex genotypes with varying degrees of environmental influences determining their expression, we have very little idea as to the contribution of recurring mutation to their frequency. There is not space here to elaborate the theoretical considerations which have been advanced. Two extreme models of population structure can be suggested. One is based on what is sometimes termed the classical view of population structure, which envisages a kind of ideal man who is homo-

[1] See also Woolf, p. 224 of this number of the Bulletin.—Ed.

zygous at every locus for the most favourable allele which occurs at that locus. It is admitted that there are a number of loci at which balanced polymorphisms exist and that homozygosity may be for different alleles in different races of men. Mutations which occurred and were favourable would gradually, over immense time if necessary, replace entirely the previous most frequent alleles at loci. However, the chance that a favourable mutation would arise is extremely small, and practically all freshly arising mutations would be unfavourable even in the heterozygous form and more unfavourable in the homozygous form. As such, at any locus where there was heterozygosity the less favourable alleles would be mutations in process of elimination. The great majority of such mutations would only individually contribute to a very slight extent to lessening of viability or fertility but sooner or later would be eliminated, being in the process detrimental to the health of those carrying it, whether eliminated after a few or many generations. On that basis all mutations must be regarded as unconditionally harmful and as determining shortening of life-span whether or not they are expressed as a recognizable disorder. If this view of population structure is accepted, then all disease other than that entirely environmentally caused would be in proportion to—and represent the "visible" proportion of—the "load" of mutations which are carried and are in process of elimination by selection. This would apply irrespective of whether specific traits were determined by single genes or by more than one acting in concert or synergistically.

These views have mostly been advanced by Muller (1950, 1956), but they are accepted by implication by Morton and by Crow (Morton, Crow & Muller, 1956; Crow, 1957) and by Kimura (1960).

At the other extreme, views of population structure have been advanced which would lead to a different conception of the part played by recurring mutations in maintaining the frequency of disorders determined in whole or part by genotype. Even the extremists would be in basic agreement about single-gene expressions, but they would regard differently the significance of recurring mutations in determining the frequency of disorders determined by more complex genotypes. Various aspects of the problems have been discussed by Lerner (1954), Waddington (1957) and Dobzhansky (1955).

In essence it is conceived that some heterozygosity has advantages, not only to populations in so far as it permits of adaptations to long-term or short-term environmental changes, but also to the individual in terms of development to "normality". This heterozygosity is maintained by various balanced advantage and disadvantage mechanisms. Even the more or less random occurrence of unfavourable gene combinations which in individuals may cause malformation or disease may be offset by the fact that these same genes may maintain their frequency in populations only because alone or in other combinations they are favourable to viability and fertility.

Clearly, on such a type of population model the relationship between recurring mutation and increase by selection in determining gene and trait frequencies must be very complex.

It would seem that in the light of our present knowledge the view taken by the United Nations Scientific Committee is the most logical. This Committee in effect takes the view that all mutations which determine individually detectable traits are harmful in any combination.

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DR C. A. CLARKE has been Consultant Physician to the United Liverpool Hospitals since 1946, and Reader in Medicine in the University of Liverpool since 1958. He qualified at Guy's Hospital, London, in 1932, and during the Second World War served as Medical Specialist in the Royal Naval Volunteer Reserve. His interest in genetics derives directly from work on butterflies begun in 1952, and this has been the subject of numerous papers in conjunction with Dr P. M. Sheppard. In human genetics his chief interest has been in the relationship between polymorphic systems and disease, and his published work includes a paper on blood groups and disease (*J. med. Educ.* 1959, 34, 400) and, jointly with other workers, papers on duodenal ulcer and the ABO blood groups (*Brit. med. J.* 1956, 2, 725) and on protective factors in erythroblastosis (*Int. Arch. Allergy*, 1958, 13, 380).

PROFESSOR W. M. DAVIDSON is Clinical Pathologist to King's College Hospital, London, and Professor of Haematology in the University of London. He has worked in pathology since 1935 and was previously Senior Lecturer in Pathology at the University of Aberdeen. During the Second World War he served in the Royal Army Medical Corps from 1939 to 1945 and was mentioned in Despatches. His research has been mainly connected with inherited disorders, and this work is continuing. The following are three of Professor Davidson's publications: "Bilateral absence of the kidneys and related congenital anomalies", with G. I. M. Ross (*J. Path. Bact.* 1954, 68, 459); "A morphological sex difference in the polymorphonuclear neutrophil leucocytes", with D. R. Smith (*Brit. med. J.* 1954, 2, 6); and "Giant neutrophil leucocytes: an inherited anomaly", with R. D. G. Milner & S. D. Lawler (*Brit. J. Haemat.* 1960, 6, 339).

DR DAVID A. PRICE EVANS has been a lecturer in internal medicine in the University of Liverpool since 1960. He qualified in medicine from the University of Liverpool in 1951 and has held various clinical appointments, has served as a Medical Specialist in the Royal Army Medical Corps, and has held a fellowship in the Department of Physiology, University of Liverpool, and also in the Division of Medical Genetics of the Department of Medicine, Johns Hopkins University School of Medicine, Baltimore. His principal research has been in the fields of peptic ulceration and the genetics of isoniazid metabolism in man, and his publications include two papers entitled "The estimation of fucose in saliva" and "The fucose and agglutinogen contents of saliva in subjects with duodenal ulcers" (*J. Lab. clin. Med.* 1960, 55, 381, 386), and, jointly with other authors, "Genetic control of isoniazid metabolism in man" (*Brit. med. J.* 1960, 2, 485), and "Further observations on the determination of the isoniazid inactivator phenotype" (*Bull. Johns Hopk. Hosp.* 1961, 108, 60).

DR C. E. FORD has been a member of the Medical Research Council's Scientific Staff since 1949. His early work had included a study of non-disjunction in *Oenothera*. Later, he investigated chromosome breakage by ionizing radiation and chemical agents. Observations begun in 1954 on reciprocal translocations in the mouse led him (jointly with J. L. Hamerton, D. W. H. Barnes and J. F. Loutit) to the proof by cytogenetic methods that bone-marrow cells injected into irradiated mice proliferate within the host and form new haemopoietic tissue. This work appeared as "Cytological identification of radiation-chimaeras" (*Nature, Lond.* 1956, 177, 452). The technical methods devised in connexion with this investigation led, in turn, to his joint work on the chromosomes of neoplastic cells and in the field of human cytogenetics. Dr Ford's other publications include: "Chromosomal changes in primary and transplanted reticular neoplasms of the mouse", with J. L. Hamerton & R. H. Mole (*J. cell. comp. Physiol.* 1958, 52, Suppl. No. 1, p. 235), and "Human somatic chromosomes", with P. A. Jacobs & L. G. Lajtha (*Nature, Lond.* 1958, 181, 1565).

DR D. G. HARNDEN has been a member of the Medical Research Council's Clinical Effects of Radiation Research Unit, Edinburgh, since 1959. He graduated in zoology from Edinburgh University in 1954 and was Assistant Lecturer in Zoology at Edinburgh before joining the Medical Research Council staff, first at the Radiobiological Research Unit, Harwell, and then in Edinburgh. Dr Harnden has worked with Dr J. M. Mitchison on the control of cell division but, since joining the Medical Research Council, his main interest has been in human cytogenetics. He developed a technique for the examination of human chromosomes in cells derived from skin biopsies (*Brit. J. exp. Path.* 1960, 41, 31). In collaboration with J. H. Edwards, A. H. Cameron, V. M. Crosse & O. H. Wolff, he described a new trisomic syndrome (*Lancet*, 1960, 1, 787), and, with P. A. Jacobs and others, a number of abnormalities of the human sex chromosomes (e.g., *Lancet*, 1960, 1, 1213).

PROFESSOR H. HARRIS's medical education was at Cambridge and Manchester; he qualified in 1943. Between 1947 and 1953, he did research work on human genetics in the Department of Biometry, Eugenics and Genetics, and in the Department of Biochemistry, at University College, London. In 1953 he moved to the Department of Biochemistry at the London Hospital Medical College, where he was Senior Lecturer and subsequently Reader in Biochemical Genetics. Since 1960 he has been Professor of Biochemistry at King's College, London. His main interest is the genetics of biochemical and metabolic variation in man. His published work includes *Human biochemical genetics* (1959), which is reviewed on p. 262 of this number of the Bulletin. Professor Harris previously contributed a paper on renal aminoaciduria to the Bulletin (*Brit. med. Bull.* 1957, 13, 26).

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DR BERNARD LENNOX is Senior Lecturer in Pathology at the Western Infirmary in the University of Glasgow. He qualified at the University of Durham. He spent much of the Second World War as a prisoner of the Japanese in Singapore and Siam, and made some observations on thiamine deficiency and Wernicke's encephalopathy during that time. From 1946 to 1955 he was Lecturer in Pathology at the Postgraduate Medical School of London. His principal interest for most of that time was in the histopathology of skin tumours, with diversions into experimental investigations of the homograft problem and other subjects. Since taking part in some of the earlier successful exploitations of the nuclear sexing technique in 1954, he has been much concerned with this subject and with chromosome counting methods. He is an assistant editor of the *Journal of Pathology and Bacteriology*, and his recent publications include chapters on histopathology of tumours and nuclear sexing, in the 7th edition of *Recent advances in pathology* (edited by C. V. Harrison, 1960), a chapter on the nuclear sex of tumours in *Die Intersexualitat* (edited by C. Overzier, 1961) and, jointly, a paper on the XXX syndrome (*Lancet*, 1960, 2, 626).

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MRS ELSIE SILK graduated in physiology at University College, London, in 1949. In 1951 she joined Dr H. Lehmann as his Research Assistant at St Bartholomew's Hospital, London. She has worked on different aspects of hospital biochemistry with the study of pseudo-cholinesterase always as her main interest, and it is with this subject that most of her publications have been concerned.

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DR L. I. WOOLF is a member of the External Scientific Staff of the Medical Research Council. He studied chemistry at University College, London, and later worked on reaction mechanisms under C. K. Ingold. From 1947 to 1951 he was an ICI Research Fellow at the Institute of Child Health, University of London, and from 1951 to 1958 held a research fellowship at The Hospital for Sick Children, Great Ormond Street, London. He has worked on some inborn errors of metabolism, the excretion of amino acids and sugars during infancy and childhood and in various diseases, the metabolism of phenylalanine and tyrosine in young infants, and leucine-sensitive hypoglycaemia. Three of his recent publications are: with H. R. Bloxam, M. G. Day & N. K. Gibbs, "An inborn defect in the metabolism of tyrosine in infants on a normal diet" (*Biochem. J.* 1960, 77, 320); "The hypoglycaemic effect of leucine and isovaleric acid" (*Clin. chim. Acta*, 1960, 5, 327); with C. O. Carter, "The birthplaces of parents and grandparents of a series of patients with phenylketonuria in south-east England" (*Ann. hum. Genet.* 1961, 25, 57). His chief interest at present is in phenylketonuria, with special reference to the relation between biochemical error and mental defect, and to the detection, treatment and genetics of the condition.

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Book Reviews

Ciba Foundation Symposium on Biochemistry of Human Genetics

Ciba Foundation. Edited by *G. E. W. Wolstenholme & Cecilia M. O'Connor.* London: J. & A. Churchill Ltd., 1959. xii+347 pages; illustrations. 21×14 cm. £2 10s.

Introduction to Quantitative Genetics

D. S. Falconer. Edinburgh: Oliver & Boyd, 1960. ix+365 pages; illustrations. 23×15 cm. £1 15s.

Human Biochemical Genetics

H. Harris. Cambridge: University Press, 1959. viii+310 pages; figures. 22×14 cm. £1 17s. 6d.

Outline of Human Genetics

L. S. Penrose. London: Heinemann, 1959. xii+146 pages; figures; plates. 19×13 cm. 12s. 6d.

Recent Advances in Human Genetics

Edited by *L. S. Penrose*, with the assistance of *Helen Lang Brown.* London: J. & A. Churchill Ltd., 1961. vii+194 pages; figures; plates. 21×14 cm. £1 7s. 6d.

Natural Selection in Human Populations

Edited by *D. F. Roberts & G. A. Harrison.* Oxford: Pergamon Press, 1959. (Symposia of the Society for the Study of Human Biology, Vol. II.) viii+76 pages; 1 plate. 22×14 cm. £1

An Introduction to Medical Genetics

J. A. Fraser Roberts. 2nd ed. London: Oxford University Press, 1959. xii+263 pages; 107 figures. 25×17 cm. £1 15s.

Human Growth

Edited by *J. M. Tanner.* Oxford: Pergamon Press, 1960. (Symposia of the Society for the Study of Human Biology, Vol. III.) vii+120 pages; figures; 1 plate. 22×14 cm. £1 10s.

This collection of books published in Great Britain in recent years covers the various disciplines which are now subsumed under the all-embracing title of "human genetics". "Human geneticists" now include exponents of many scientific techniques and, indeed, this is only natural, for the study of inheritance may clearly be extended to any biological function. At the beginning of this century, while a corpus of theory already existed, methods were lacking; but this situation is being remedied and the new approaches are amply reflected in these books.

Recent advances in human genetics may serve as a framework within which to discuss the other volumes. It still concerns itself to a great extent with the ideas and methods of earlier days. Dermal ridge-patterns, treated by S. B. Holt, were one of the classical quantitative characters analysed by many investigators, beginning with Galton. We are still no nearer an understanding of the mechanism of the formation of these patterns. When this is elucidated, it may be expected that a knowledge of the segregating genes involved will follow.

Introduction to quantitative genetics discusses very expertly the mathematical techniques and theories which are of use in dealing with this most difficult subject. While the author's interests lie in work on experimental animals, and the applications of this type of research lie mainly in agriculture, none the less inheritance of metrical characters is also of very great importance in human genetics. Many of our most important qualities, such as intelligence, viability, fertility and resistance to infection, are metrical. Our social structure and our evolution depend on these qualities; and it may well be that when it is possible to apply the techniques described in this book—and newer ones—to man as well as to the mouse and *Drosophila*, problems of quantitative inheritance in man will reassume the importance they held in the hands of pioneers such as Galton and Pearson. One such quantitative character treated by Penrose in *Recent advances in human genetics* is the growth of the normal foetus. In the second part of this chapter he attempts to analyse the causes of the processes in abnormal foetal growth—the very large, and as yet mysterious, subject of congenital malformations. In some cases a simple genetic background can be established. In others, extrinsic prenatal causes such as radiation or maternal rubella are implicated. Recent discoveries have shown that chromosomal aberrations are another common aetiological factor. In a large number of congenital malformations, however, quantitative inheritance is again of great importance.

Human growth is also the subject, and title, of volume III of the Symposia of the Society for the Study of Human Biology. Postnatal changes with age in primates and man are considered by A. H. Schultz. W. J. Israelsohn discusses methods of analysis of growth. J. M. Tanner writes on the genetic control of human growth and the influence of sex. D. F. Roberts describes the influence of race and climate. Environmental factors such as nutrition and disease are reviewed in a paper by R. M. Acheson, and T. McKeown reverts to the subject of prenatal growth. The effect of the environment is nowhere shown more clearly than in the very marked secular changes in stature and intelligence which have been observed and which are described in this volume by A. W. Boyne.

Since the pioneer work of Darwin, the genetic bases of natural selection and evolution have been much studied. This theoretical approach is not neglected in the books included in this review. Thus, a paper by Penrose on mutation, in *Recent advances in human genetics*, is particularly topical in view of the well-justified alarm about the effects of radiation. Falconer's book, *Introduction to quantitative genetics*, is to a large extent concerned with evolutionary theory, and volume II of the Symposia of the Society for the Study of Human Biology is entitled *Natural selection in human populations*. This volume contains, as well as an introductory paper by Penrose, a discussion of mathematical models for selection, by A. R. G. Owen, the relative fitness of mutant genes, by C. A. Clarke, and human polymorphisms, by P. M. Sheppard. In addition, T. Dobzhansky has contributed a paper embodying the results of his fundamental work on selection of gene systems in natural populations in *Drosophila*. Finally, E. H. Ashton discusses primate evolution as a whole.

In very recent years there have opened up two approaches which have revolutionized the study of Mendelian segregating traits in man. These are the adaptation of old, and the development of new, biochemical and cytological techniques to deal with problems of human genetics. In *Recent advances in human genetics*, P. S. Gerald discusses the abnormal haemoglobins. The discovery of variants of this very important molecule and the characterization of differences between them have opened up new vistas in the elucidation of the chain of events between the gene and the protein whose production it controls. Harris's book, *Human biochemical genetics*, and the *Ciba Foundation Symposium on Biochemistry of Human Genetics*, are excellent and instructive elaborations of this central theme. In some cases we are nearer the understanding of this chain of events than in others; in all cases our primary concern is to uncover its ramifications. While many of the traits described are of less social importance than common diseases and quantitative traits such as intelligence and disease resistance, it is already a far cry from A. E. Garrod's original work, *Inborn errors of metabolism*, to the beautiful studies of gene action and interaction described in these volumes. Harris's work is a complete review of the present state of our knowledge. The Ciba Foundation Symposium is a

collection of first-hand reports on the researches of the most eminent workers in this field.

The last two papers in *Recent advances in human genetics* are a contribution on linkage and a general review of statistical methods and theory, by J. H. Renwick and C. A. B. Smith, respectively. Both these chapters deal with subjects of primary importance in the application of genetic methods to medicine. They find their place also in the second edition of J. A. Fraser Roberts' well-known book, *An introduction to medical genetics*. This is indeed an excellent introduction for all medical men to the problems of human genetics and to their importance in the practice of medicine. While at the moment our understanding of the inheritance of pathological conditions is restricted to a rather esoteric galaxy, there is every prospect that the genetic basis of more common diseases will be elucidated in the near future and will add much to our therapeutic resources. Indeed, even the conditions we do understand, while individually rare, are, as this book shows, so numerous that their totality represents a not unimportant proportion of human pathology.

It was barely five years ago that it became possible to examine human mitotic chromosomes, and only two years ago that workers in France, England and Sweden showed almost simultaneously that mongolism, a common and baffling disease, was frequently associated with trisomy of one of the smaller chromosomes. Since that time, this subject has been extensively and enthusiastically developed, and has already contributed significantly to our understanding of much congenital disease and malformation. It is too early for any synoptic views of this already very large subject, but in *Recent advances in human genetics* two chapters are devoted to it. One of the pioneers in this field, D. G. Harnden, reviews what is known of the normal chromosome complement and also discusses the abnormalities discovered up to early 1960. O. J. Miller has expanded the subject of abnormalities of the allosomes or sex chromosomes, and has in addition reviewed other mechanisms of deviations of sexual development.

Finally, in a creditably short volume, *Outline of human genetics*, Penrose has attempted to draw these various threads together from the point of view of the human biologist rather than the medical man. Devoting approximately a score of pages to each, he reviews in turn basic theories of inheritance as applied to problems in man, single-gene effects, population genetics, association and linkage, and the interaction of environment and heredity. The book appeared just in time to include in this last section a brief reference to chromosomal aberrations.

From the point of view of the medical practitioner, it is of importance to advise persons who contemplate marriage or who, having already had children affected with some genetically determined complaint, wish to have others. These problems of eugenics and genetic prognosis or counselling are fraught with scientific and ethical difficulties. Both Penrose and Fraser Roberts attempt to deal with these in the last chapters of their books, the former primarily from the theoretical view-point, the latter more practically. In both cases the conclusions are commendably cautious. The time for control of matings and child-bearing is not yet; both the knowledge and the moral basis are lacking. *G. R. Fraser*

The Hazards to Man of Nuclear and Allied Radiations: a Second Report to the Medical Research Council

Medical Research Council. London: Her Majesty's Stationery Office, 1960. (Cmd. 1225) vii + 154 pages; figures. 24 × 15 cm. 7s.

The first Report of the committee appointed by the Medical Research Council to consider the medical and genetic aspects of nuclear radiation was presented to Parliament in June 1956 (Cmd. 9780). The second Report was the result of a further meeting of the committee in 1959. During the intervening months, the subject was kept under review by various standing committees of the Council, and a number of other reports were published, including those by the United States National Academy of Sciences and by the United Nations Scientific Committee on the Effects of Atomic Radiation.

The second Report contains five chapters, in addition to the introduction, and covers the types of radiation and their biological effects, genetic effects of radiation, effects of radiation on the health of the individual, levels of exposure to radiation, and assessment of the hazards of exposure. There are 11 appendices by specialist workers, which have provided much of the technical information on which the Report is based.

In the chapter on genetic effects of radiation, the committee point out that rapid advances have recently been made in the knowledge of human chromosomes—their number, their characteristic features and functions, and the occurrence of modifications of their normal patterns. These advances have called for correction on points of detail in the text of the first Report; nevertheless, the committee do not wish to alter materially the views they expressed in that Report. *O.M.H.*

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"'A conspicuous feature of modern medical research has been the increasing interest in the role of genetic factors in disease.' This quotation is from the Introduction by Sir Charles Harington to this impressive symposium on *The Thyroid Gland*. The fifteen articles which follow on various aspects of thyroid function in health and in disease certainly bear out this pronouncement. No less than three are directly concerned with genetics; in most of the other twelve exciting hints abound of how genetically determined polymorphisms may be implicated in the pathogenesis of thyroid disease." (*Annals of Human Genetics*, 1960, 24, 393.)

Hypothermia and the Effects of Cold (Vol. 17, No. 1; January, 1961)

". . . the extensive review of the whole field to which the latest issue of the *British Medical Bulletin* is devoted under the editorship of Dr. A. S. Parkes, F.R.S., serves a most valuable purpose. As a source of up-to-date information on progress in medicine it has a practical use; to the non-expert the 15 articles written by acknowledged authorities offer a fascinating report on man's inventiveness." (*British Medical Journal*, 1961, 1, 567.)

The contents of the next two numbers—GENETICS OF MICRO-ORGANISMS—THE ADRENAL CORTEX—are listed on the back cover of this issue.

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